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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(54) Title: POLY(DIPEPTIDE) AS A DRUG CARRIER

(57) Abstract

A novel polypeptide drug carrier is provided wherein polypeptides containing glutamic acid and aspartic acid, or glutamic acid/alanine, or glutamic acid/asparagine, or glutamic acid/glutamine, or glutamic acid/glycine, are conjugated to drugs in order to improve the solubility of the drugs and/or their therapeutic efficacy *in vivo.* An illustrative example involves the conjugation of paclitaxel to a poly(glutamic acid/aspartic acid) polypeptide and its efficacy in the treatment of prostate cancer *in vivo.*

POLY(DIPEPTIDE) AS A DRUG CARRIER

SPECIFICATION

Field of the Invention

The present invention relates to novel drug carriers and their use. More particularly, the present invention relates to the novel use of a poly(di-peptide) peptide covalently bound to a drug to act as a drug carrier, particularly for poorly water soluble drugs. The poly(dipeptide) may be

5   composed of a combination of glutamic acid and aspartic acid. The poly(dipeptide) may be composed of combinations of glutamic acid with alanine, asparagine, glycine or glutamine.

Background of the Invention

It is undisputed that advances in pharmaceuticals have revolutionized health care for humans

10   and other animals as well. However, despite the outstanding advances made in the field of pharmacology, some significant limitations still remain in the treatment of various diseases via drug agents. One of the most significant limitations at this time relates to the delivery of particular drugs *in vivo*, especially in situations where drugs are poorly water soluble. Indeed, the use of some drugs which show great promise *in vitro*, has been severely limited due to issues related to their solubility.

15   This causes problems with drug delivery *in vivo*. One example of such a drug is paclitaxel in the treatment of tumors, especially for example, in the case of prostate cancer.

As discussed below, the prior art has attempted to address this issue in a number of ways. However, as presented in more detail below, prior to the instant invention, the unique advantages of conjugating a drug to the inventive polypeptide, while desired, were unknown.

20   United States Patent No. 4,675,381, issued to Bichon, on June 23, 1987, entitled "Biodegradable Polypeptide and its Use for the Gradual Release of Drugs," discloses a polyaspartate and/or polyglutamate polymer as a drug carrier. This patent envisions the use of polyaspartate and/or polyglutamate polymers as drug carriers wherein the drug is encapsulated or incorporated in the matrix of the polymer. The patent does not disclose, teach or suggest covalent conjugates of the

25   drug with the polymer. Furthermore, most of the teaching in the patent is directed to homopolymers of aspartate and glutamate, not combinations of the two amino acids.

United States Patent No. 5,087,616 issued to Myers et al. on February 11, 1992, entitled "Cytotoxic Drug Conjugates and Their Delivery to Tumor Cells," discloses the use of a biodegradable polymeric carrier to which one or more cytotoxic molecules, for instance, daunomycin

is conjugated. The biodegradable polymeric carrier is specified to be, for example, a homopolymer of polyglutamic acid. However, the use of a drug conjugated to a di-peptide copolymer carrier is clearly not disclosed, taught or suggested in this reference.

A 1983 *J. Med. Chem.* paper by Piper et al. entitled "A Synthetic Approach to Poly(γ-
5   glutamyl) Conjugates of Methotrexate" discloses the use of methotrexate conjugated to 2 to 3 glutamic acid units. This paper does not disclose, teach or suggest di-peptide polymers of glutamic acid and aspartic acid, or glutamic acid with alanine, asparagine, glutamine or glycine.

A 1982 *Int. J. Cancer* paper by Zunino et al. entitled "Anti-Tumor Activity of Daunorubicin Linked to Poly-L-Aspartic Acid" discloses daunorubicin bound to a homopolymer of polyaspartic
10   acid. The paper indicates that "the binding (of daunorubicin) to the polypeptide markedly reduced drug toxicity but only slightly decreased drug potency." "The daunorubicin-poly-L-aspartic acid conjugate demonstrated anti-tumor activity comparable to that of doxorubicin in leukemia models, but superior to that of doxorubicin in a solid tumor model." While this paper does disclose the covalent conjugation of an anti-tumor drug to a homopolymer of polyaspartic acid, it does not
15   disclose, teach or suggest the use of a di-peptide containing polymer of aspartic acid and glutamic acid, or glutamic acid with alanine, asparagine, glutamine or glycine.

A 1998 *Cancer Research* paper by Li et al. entitled "Complete Regression of Well-established Tumors Using a Novel Water-soluble Poly(L-Glutamic Acid)-Paclitaxel Conjugate," discloses the use of a water-soluble poly-L-glutamic acid-paclitaxel conjugate to produce tumor
20   effects with diminished toxicity. However, this paper does not disclose, teach or suggest the use of the inventive copolymer.

A 1989 *J. Pharm. Exp. Ther.* paper by Ramsammy entitled "Polyaspartic Acid Protects Against Gentamicin Nephrotoxicity in the Rat," discloses the use of poly-amino acids, including polyaspartic acid, to provide protection against the development of amino glycoside-induced
25   nephrotoxicity in the rat. However, this paper does not disclose, teach or suggest the inventive copolymer, much less the inventive copolymer conjugated to a drug.

A 1990 *Biopolymers* paper by Hayashi and Iwatsuki, entitled "Biodegradation of Copoly(L-Aspartic Acid/L-Glutamic Acid) *In Vitro*," discloses the preparation of copolypeptides consisting of L-aspartic acid and L-glutamic acid. The paper describes the use of such polypeptides to
30   determine the effects of copolymer composition and sequential distributions on the rate of degradation by papain to stimulate *in vivo* polymer degradation. This paper does disclose, teach or

suggest the use of copolymers of glutamic acid and aspartic acid, similar to the copolymer of the present invention. The paper also does not disclose, teach or suggest the use of such copolymers covalently conjugated with drugs.

5   United States Patent No. 4,960,790 issued to Stella et al., and entitled "Derivatives of Taxol, Pharmaceutical Compositions Thereof and Methods for the Preparation Thereof" discloses the anti-tumor agent taxol covalently conjugated with, for example, an amino acid (for example, glutamic acid). However, this patent does not disclose, teach or suggest the use of large polypeptides, much less the use of the inventive polypeptide containing glutamic acid and aspartic acid, or glutamic acid with alanine, asparagine, glutamine or glycine.

10   Finally, a 1960 *J. Am. Chem. Soc.* by Karlson et al., entitled "The Helical Sense of Poly-β-benzyl-L-aspartate" discusses the physical characteristics of series of copolymers derived from γ-benzyl-L-glutamate and β-benzyl-L-aspartate. However, this paper does not disclose, teach or suggest the use of such copolymers *in vivo* much less their conjugation with anti-tumor drugs.

        As indicated from the above art, there has been a long-felt need in the art to attempt to
15 solubilize poorly soluble drugs, such as anti-tumor agents, and in this endeavor, the art has attempted to accomplish this by various means, including the use of, for example, polypeptides comprising homopolymers of poly-glutamic acid and poly-aspartic acid. However, as discussed in more detail below, the present inventive di-peptides conjugated to drugs to increase their solubility *in vivo*, while desired in the art, has not been anticipated or suggested by the art.

20   In order to demonstrate one embodiment of the present invention, a conjugate of the antitumor agent paclitaxel was made with the inventive polypeptide (for example, a poly(dipeptide) comprising glutamic acid and aspartic acid) and used as a drug delivery vehicle. It was then shown that this inventive conjugate possessed superior biological and therapeutic properties *in vivo* over, for example, unconjugated drug, and the drug conjugated with known prior art carriers (*e.g.*,
25 homopolymers of glutamic acid and aspartic acid). Data below shows that, for example, conjugating the antitumor drug paclitaxel to the inventive polymer–and only the inventive polymer– results in unexpected therapeutic properties of paclitaxel such as the treatment of prostate cancer. Indeed, the following results show what applicants believe is the first described efficacy of paclitaxel in any form against prostate tumors *in vivo*.

30   Paclitaxel was selected for an exemplary embodiment of a drug to be conjugated with the inventive carrier as an example of the practice of the instant invention because paclitaxel is a known

antitumor drug, with known solubility problems *in vivo*. Hence, it has known effectiveness problems and toxicity problems *in vivo* related to its stability and related *in vivo* use. Furthermore, the need for the present invention (*i.e.*, a carrier that can solubilize and/or enhance the *in vivo* therapeutic use of drugs, for example, poorly soluble drugs, such as, for example, poorly soluble

5 antitumor drugs) is amply demonstrated by paclitaxel since the prior art has made a number of attempts at conjugating the drug to various carriers, including polypeptides, in order to improve the biological use (see above, Background of the Invention).

Paxlitexel is an antitumor agent that works as an antitumin agent. Improvements of cancer treatment is extensively determined by the development of more tumor specific pharmaceuticals and

10 new drug techniques. Due to an angiogenesis process involved in the tumor vascular density, antitubular agents have opened a new era in the treatment of various tumors and have undergone extensive preclinical development and evaluation.

Tubulin is a principal protein of subunit of microtubulars. Microtubulars assemble when they are required by a cell for a particular function and depolymerize when they are no longer

15 needed. Therefore, tubulin is a cellular target for antimitotic agents. Some of these agents, such as vincristine, vinblastine, rhizoxin, maytansin and epipodophyllotoxins interact with tubulin on the colchicine binding sites to inhibit tubular polymerization and thereby cause cellar rest metephase. Paclitaxel on the other hand, acts to promote assembly of micotubulars resulting in highly stable but non-functional polymers that lead to mitotic for rest of poliferating cells. Schiff, P.B., Horwitz, S.B.

20 *Proc. Natl. Acad. Sci. USA*. **1980**, 77, 1561-1565; Schiff, P.B., Fant, J., Horwitz, S.B. *Nature (London)* **1980**, 283, 665-667; Rowinsky, E.K., Cazenave, L.A., Donehower, R.C. *JCNI, J. Natl Cancer Inst.* **1990**, 82, 1247-1259, Imbert, T.F. *Biochimie*, **1998**, 80; 207-222; Sandler, E.S., Friedman, D.J., Mustafa, M.M., Winick, N.J., Bowman, W.P.,Buchanan, G.R. *Cancer* **1997** 79(5), 1049-1054.

25 In the art, paclitaxel formulated in the cremophor has been used to treat breast, ovarian, colon and lung cancers (Rowinsky, E.K.; Donehower, R.C. *Cancer Res.* **1998**, 58, 2404-2409; Holmes, F.A., Kudelka, A.P., Kavanagh, J.J., Huber, M.H., Ajani, J.A., Valero, V. *In: G.I. Georg. T.T. Chen I. Ojima and D.M. Vyas (eds)*. **1995**, 31-57; Cortes, J.E.; Padur,, R.J., *Clin. Oncology*. **1995**, 13(10), 2643-2655).

30 However, despite some effectiveness of paclitaxel, there are notable side effects such as granulocytopenia and body weight loss (Rowinsky, E.K. and Donehower, R.C., Review: Paclitaxel

(Taxol), *New Eng. J. Med.* 1995, 332, 1004-1014). It is well known in the art that the poor water solubility of paclitaxel makes the drug difficult to administer intraveneously.

Furthermore, and importantly, it is known in the art that paclitaxel, while showing some effectiveness in the breast and ovarian cancers, is not effective in the treatment of prostate cancer.

5   Paclitaxel was, therefore, chosen, as a exemplary drug in which to conjugate to the inventive di-glutamic acid/aspartic acid polypeptide in order to determine whether conjugation in the inventive complex produces a drug carrier complex which shows improved therapeutic use. As discussed below, this is indeed the case, and only when paclitaxel is conjugated to the inventive conjugate has it ever been shown to be effective *in vivo* against prostate cancer.

10

Summary of the Invention

An object of the invention is the provision of a therapeutic compound including a drug carrier.

An object of the invention additionally is a method for improving the solubility of a drug 15   moiety.

An additional object of the invention is a method for treating a condition.

Thus, in accomplishing the foregoing objectives, there is provided in accordance with one aspect of the present invention a therapeutic compound comprising at least one drug moiety, and at least one polypeptide drug carrier moiety, the drug moiety being covalently linked to the carrier 20   moiety, and the polypeptide drug carrier moiety comprising glutamic acid and a second amino acid selected from the group consisting of aspartic acid, alanine, asparagine, glutamine, glycine, and combinations of two or more amino acids selected from the group consisting of aspartic acid, alanine, asparagine, glutamine, and glycine.

In a specific embodiment, the second amino acid is aspartic acid, the drug moiety is selected 25   from the group consisting of anti-tumor drugs, cardiovascular drugs, anti-microbial drugs, diabetic drugs, anti-inflammatory drugs, and pain alleviating drugs.

In a specific embodiment, the drug moiety is selected from the group of drugs consisting of, for example, paclitaxel, epipodophyllotoxin, vincristine, docetaxel, daunomycin, doxorubicin, mitoxantrone, topotecan, bleomycin, gemcitabine, fludarabine and 5-FUDR.

30   In a preferred embodiment, the drug moiety is paclitaxel.

In a specific embodiment, the polypeptide drug carrier moiety comprises from about 50 to about 90 percent, by weight, glutamic acid, and from about 10 to about 50 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof, more preferably from about 60 to about 80 percent, by weight, glutamic acid, and from about 20 to about 40 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof, and most preferably from about 70 to about 75 percent, by weight, glutamic acid, and from about 25 to about 30 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof.

In another embodiment, the therapeutic compound comprises at least two drug moieties, which may not the same as each other.

In another embodiment, the therapeutic compound comprises a plurality of drug moieties.

In still another embodiment, the drug moiety of the therapeutic compound comprises from about 10 percent to about 60 percent, by weight, more preferably from about 20 percent to about 50 percent, by weight, and most preferably from about 20 percent to about 40 percent, by weight of the therapeutic compound. Moreover, the polypeptide drug carrier moiety may comprise from about 40 percent to about 90 percent, by weight, more preferably from about 50 percent to about 80 percent, by weight, and most preferably from about 60 percent to about 80 percent, by weight of the therapeutic compound.

In preferred embodiments (for example, paclitaxel with a poly(dipeptide) glutamic acid/aspartic acid carrier), the drug moiety does not comprise more than about 60% by weight of the therapeutic compound (in order to not adversely affect solubility and/or viscosity which can effect injectability of the compound).

In a preferred embodiment, the drug moiety is paclitaxel, the carrier moiety comprises about 70 percent glutamic acid and about 30 percent aspartic acid, the paclitaxel drug moiety is about 20 percent to about 40 percent, by weight, of the therapeutic compound, and the molecular weight of the therapeutic compound is from about 20,000 to about 50,000 daltons.

Further in accomplishing the foregoing objectives, there is provided in accordance with another aspect of the present invention a method for improving the solubility of a drug moiety comprising the steps of covalently conjugating at least one drug moiety with at least one polypeptide drug carrier moiety, thereby creating a therapeutic compound, the therapeutic compound comprising at least one drug moiety, and at least one polypeptide drug carrier moiety, the drug moiety being

covalently linked to the carrier moiety, and the polypeptide drug carrier moiety comprising glutamic acid and aspartic acid or alanine, or asparagine, or glutamine, or glycine, or combinations thereof.

In a preferred embodiment, the water solubility of the therapeutic compound is greater than the water solubility of the drug moiety.

5   In another embodiment, the drug moiety is an antitumor drug.

In a preferred embodiment, the drug moiety is paclitaxel.

In a specific embodiment, the polypeptide drug carrier moiety comprises from about 50 to about 90 percent, by weight, glutamic acid, more preferably from about 60 to about 80 percent, by weight, glutamic acid, and most preferably from about 70 to about 75 percent, by weight, glutamic

10   acid, and from about 10 to about 50 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine or combinations thereof, more preferably from about 20 to about 40 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof, and most preferably from about 25 to about 30 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof.

15   Further regarding the role of alanine, asparagine, glutamine, and/or glycine in the present invention, the following is noted. It is believed at the time of the application that a preferred embodiment uses a poly(dipeptide) polymer of glutamic acid and aspartic acid. However, it is also believed, but not in any limiting sense, that any amino acids similar to aspartic acid, including alanine, asparagine, glutamine, and glycine, can be substituted for aspartic acid in the inventive

20   poly(dipeptide). While not wishing to be bound in anyway, at the time of the application it is believed that a key aspect of the inventive poly(dipeptide) relates to the glutamic acid backbone. It is believed that as long as glutamic acid is present in the poly(dipeptide), aspartic acid may serve as the other amino acid, or any amino acid similar to aspartic acid, such as, for example, alanine, asparagine, glutamine, and glycine may be used. These amino acids may be substituted in whole

25   or in part for aspartic acid and may be mixed. Giving a plurality of inventive poly(dipeptide) polymers, each having glutamic acid, and otherwise containing aspartic acid, or alanine, or asparagine, or glutamine, or glycine or any combinations thereof.

Further in accomplishing the foregoing objectives, there is provided in accordance with another aspect of the present invention a method for treating a condition comprising the steps of

30   administering a therapeutically effective amount of a therapeutic compound comprising at least one drug moiety, and at least one polypeptide drug carrier moiety, the drug moiety being covalently

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linked to the carrier moiety, and the polypeptide drug carrier moiety comprising glutamic acid and a second amino acid selected from the group consisting of aspartic acid, alanine, asparagine, glutamine, glycine, and combinations of aspartic acid, alanine, asparagine, glutamine, and glycine.

In a specific embodiment, the drug moiety is selected from the group consisting of anti-tumor

5   drugs, including, for example, paclitaxel, epipodophyllotoxin, vincristine, docetaxel, daunomycin, doxorubicin, mitoxantrone, topotecan, bleomycin, gemcitabine, fludarabine and 5-FUDR.

In an embodiment, the polypeptide drug carrier moiety comprises from about 50 to about 90 percent, by weight, glutamic acid, more preferably from about 60 to about 80 percent, by weight, glutamic acid, and most preferably from about 70 to about 75 percent, by weight, glutamic acid, and

10   from about 10 to about 50 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof, more preferably from about 20 to about 40 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof, and most preferably from about 25 to about 30 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof.

15   In a preferred embodiment, the condition is a prostate tumor and the therapeutic agent is paclitaxel.

Further still, in summary, the present invention relates to the discovery that a particular polypeptide composed of glutamate and aspartate makes an unexpectedly good carrier for delivery of drugs, including poorly soluble drugs. An illustrative example includes anti-tumor agents. More

20   particularly, and for example, the present invention relates to the synthesis and use of a poly (glutamate/aspartate) peptide of approximately 26,000-30,000 molecular weight, containing approximately 70% glutamic acid and 30% aspartic acid, covalently linked with a drug. Such drug may be, for example, a poorly soluble drug and/or an anti-tumor agent. One example of a preferred embodiment is the conjugation of the anti-tumor drug paclitaxel. In a preferred embodiment, the

25   concentration of the conjugated drug, for example, paclitaxel, may be from approximately 20% to 40% by weight of the overall conjugate.

As described in more detail below, the present inventors have discovered that the use of the instant inventive conjugate (poly-glutamate/aspartate polypeptide and poly-glutamate/alanine, asparagine, glutamine, glycine) results in unexpectedly good *in vivo* properties when covalently

30   linked to drugs. These properties are superior to that found for the conjugation of drugs to other drug carriers known in the art, such as other polypeptides, including homopolymers of glutamic acid

and aspartic acid. In particular, and for example, one use of the instant invention involves the conjugation of paclitaxel to the inventive peptide to enable the effective *in vivo* treatment of prostate cancer. As shown in more detail below, conjugation of paclitaxel known prior art polypeptide carriers, or use of unconjugated paclitaxel, results in ineffective treatment of prostate cancer *in vivo*.

5   However, conjugation of paclitaxel to the unique inventive copolymer results in the first ever observed therapeutic treatment of prostate cancer in animals.

Brief Description of the Drawings

Figure 1A shows a schematic of the synthesis of the inventive polypeptide

10   poly(glutamic/aspartic acid).

Figure 1B shows a sample amino acid analysis of a sample of the inventive poly(glutamic/aspartic acid) di-peptide.

Figure 2A shows a synthetic scheme conjugating paclitaxel to poly(glutamate/aspartate).

Figure 2B shows an NMR spectra of the inventive copolymer poly(glutamate/aspartate).

15   Figure 2C shows an NMR spectra of unconjugated paclitaxel.

Figure 2D shows NMR specra of an inventive conjugate of paclitaxel-poly(glutamate/aspartate).

Figure 3A shows a UV scan of unconjugated paclitaxel.

Figure 3B shows a UV scan of paclitaxel in the inventive conjugate (*i.e.*, paclitaxel

20   conjugated to poly(glutamate/aspartate)).

Figure 4A shows a UV scan of the inventive polypeptide conjugated to paclitaxel (paclitaxel-poly(glutamate/aspartate)).

Figure 4B shows a UV scan of the inventive polypeptide (poly(glutamate/aspartate)) in unconjugated form.

25   Figure 5A shows a UV scan standard curve for unconjugated paclitaxel.

Figure 5B shows a UV scan of paclitaxel conjugated to poly(glutamate/aspartate).

Figure 6 shows an HPLC analysis of paclitaxel.

Figure 7 shows an HPLC analysis of a sample paclitaxel-poly(glutamate/aspartate).

Figure 8 shows an HPLC analysis of an unconjugated inventive di-peptide

30   (poly(glutamate/aspartate)).

Figure 9A shows an HPLC chromatogram of a mixture of a poly(glutamate/aspartate)-paclitaxel conjugate and unconjugated paclitaxel.

Figure 9B shows an HPLC 3D chromatogram of a paclitaxel-poly(glutamate/aspartate) polypeptide.

5   Figure 9C shows an HPLC 3D chromatogram of a mixture of unconjugated paclitaxel and the inventive paclitaxel-poly(glutamate/aspartate acid) polypeptide conjugate.

Figure 9D shows the sustained release properties of a poly(glutamate/aspartate) acid-paclitaxel conjugate.

Figure 9E shows a useful-life determination of a paclitaxel-poly(glutamic/aspartic acid)
10   conjugate.

Figure 9F shows a *in vitro* cell culture assay of paclitaxel and the conjugate.

Figure 9G shows cytotoxicity (IC-50) of the conjugate and of paclitaxel on human prostate cancer cells (PC3) *in vitro*.

Figure 10 shows *in vivo* antitumor activity of an inventive paclitaxel-
15   poly(glutamate/aspartate) conjugate compared to unconjugated paclitaxel against mice bearing ovarian tumor.

Figure 11 shows *in vivo* antitumor activity of paclitaxel-polyglutamic acid (homopolymer) and unconjugated paclitaxel *in vivo* in mice against ovarian tumor.

Figure 12 shows *in vivo* antitumor activity of paclitaxel conjugated with poly(glutamic acid)
20   and unconjugated paclitaxel in nude mice bearing human breast cancer.

Figure 13A shows the *in vivo* antitumor activity of an inventive paclitaxel-poly(glutamic acid/aspartic acid) polypeptide and unconjugated paclitaxel in nude mice bearing human prostate cancer.

Figure 13B shows the *in vivo* antitumor activity of paclitaxel conjugated to polyglutamic acid
25   homopolymer and unconjugated paclitaxel in nude mice bearing human prostate cancer.

Figure 14 shows *in vivo* antitumor activity of paclitaxel-poly(glutamic acid/aspartic acid) conjugate, paclitaxel-poly glutamate (homopolymer) conjugate, paclitaxel-polyaspartic (homopolymer) conjugate and unconjugated paclitaxel in nude mice bearing human prostate cancer.

Figure 15A shows the antitumor activity of paclitaxel conjugated to the inventive di-peptide
30   poly(glutamic acid/aspartic acid) compared to unconjugated taxol in breast tumor-bearing athymic nude mice at 15 days post treatment.

Figure 15B shows antitumor activity of poly(glutamic acid/aspartic acid) conjugated to paclitaxel, compared to unconjugated paclitaxel in nude mice bearing human breast tumor at 43 days post treatment.

Figure 16 shows antitumor activity of paclitaxel conjugated to poly(aspartic acid/glutamic acid) compared with unconjugated paxol in prostate tumor-bearing athymic nude mice at 15 day post treatment.

Figure 17A shows antitumor activity of paclitaxel conjugated with poly(glutamic acid/aspartic acid) compared with unconjugated paclitaxel and paclitaxel conjugated with polyglutamic acid homopolymer on nude mice bearing human prostate tumor (mice at 48 hours post treatment).

Figure 17B shows antitumor activity of paclitaxel conjugated with poly(glutamic acid/aspartic acid) compared with unconjugated paclitaxel and paclitaxel conjugated with polyglutamic acid homopolymer on nude mice bearing human prostate tumor (mice at 7 days post-treatment).

Figure 17C shows antitumor activity of paclitaxel conjugated with poly(glutamic acid/aspartic acid) compared with unconjugated paclitaxel and paclitaxel conjugated with polyglutamic acid homopolymer on nude mice bearing human prostate tumor (mice at 22 days post-treatment).

The drawings are not necessarily to scale, and certain features of the invention may be exaggerated in scale or shown in schematic form in the interest of clarity and conciseness.

Detailed Description of the Preferred Embodiment

It is readily apparent to one skilled in the art that various substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

The term "therapeutic" as used here, for example, in the terms "therapeutic compound" and "therapeutically effective amount" means to have at least some minimal physiological effect. For example, a "therapeutic compound" would have at least some minimal physiological effect upon being administered to a living body. An agent may have at least some minimal physiological effect upon administration to a living body if, for example, its presence results in a change in the physiology of a recipient animal. For example, a physiological effect upon administering a

"therapeutic" anti-tumor compound may be the inhibition of tumor growth, or decrease in tumor size, or prevention reoccurrence of the tumor. Administration of a "therapeutically effective amount" means the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a change in the physiology of a recipient animal. For example, in the treatment of cancer or neoplastic disease, a compound which inhibits the growth of a tumor or decreased the size of the tumor or prevents the reoccurrence of the tumor would be considered therapeutically effective.

The term "anti-tumor drug" means any therapeutic agent having therapeutic effect against a tumor, neoplastic disease or cancer.

The term "drug" means any agent having a therapeutic effect when administered to an animal.

The dosage of the present administration for therapeutic treatment will be sufficient to generate a therapeutically effective amount of the administered agent.

The term "condition" means any condition, state, disease, abnormality, imbalance, malady and the like in an animal which one seeks to effect by administrating a therapeutically effective amount of a therapeutic compound. A condition may include, but is not limited to, cancers, neoplastic diseases, tumors, and conditions of the prostate, including prostate tumors and/or prostate cancer.

The term "treating", used for example in the term "treating a condition", means at least the administration of a therapeutically effective amount of a therapeutic compound to elicit a therapeutic effect. It does not necessarily imply "curing", but rather having at least some minimal physiological effect upon a condition upon administration to a living body having a condition. For example, treatment could encompass administering an agent and the presence of that agent resulting in a change in the physiology of a recipient animal.

The terms "peptide", "polypeptide", "di-peptide", "copolymer", "poly(glutamic acid/aspartic acid)" (and all variations thereupon) , and "inventive peptide" can refer to the peptide of the present invention as further defined herein (and comprising, for example, a polypeptide comprising aspartic acid and glutamic acid and/or polypeptides comprising aspartic acid with alanine, asparagine, glutamine and glycine, in any combination).

Dosage and Formulation

The therapeutic compounds (including compounds, drugs, conjugates and the like) of this invention can be formulated and administered to treat a variety of conditions. They can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic active ingredients or in a combination of therapeutic active ingredients. They can be administered alone, or with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice.

The dosages are determined for the chosen therapeutic use, including the condition to be treated, the therapeutic agent used to treat the condition and the type of animal treated (including considerations as to age, weight, sex and so forth). Such determinations are well within the scope of those skilled in the art and do not involve undue experimentation or exercise of inventive skill.

The dosage administered will be a therapeutically effective amount of active ingredient and will, of course, vary depending upon known factors such as a the pharmacodynamic characteristics of the particular active ingredient and its mode and route of administration; age, sex, health and weight of the recipient; nature and extent of symptoms; kind of concurrent treatment, frequency of treatment and the effect desired. Usually a daily dosage (therapeutic effective amount) of active ingredient can be about 1 to 400 milligrams per kilogram of body weight. Ordinarily, 1 to 200, and preferably 1 to 50, milligram per kilogram per day given in dividend doses 2 to4 times a day or in sustained release form is effective to obtain desired results.

Dosage forms (compositions) suitable for internal administration contain from about 1.0 to about 500 milligrams of active ingredient per unit. In these pharmaceutical compositions, the active ingredient will ordinarily be present in an amount of about 0.05-95% by weight based on the total weight of the composition.

Administration may be by any means suitable for the condition to be treated and may include, for example, oral administration. Such determination is within the ordinary level of skill of one skilled in the art. For example, oral administration may be accomplished using solid dosage forms such as capsules, tablets and powders, or in liquid dosage forms such as elixirs, syrups, emulsions and suspensions. The therapeutic compound (agent or the like) may also be, for example, parenterally administered by injection, rapid infusion, nasopharyngeal adsorption of dermoabsorption. The agent may also be administered intramuscularly, intravenously, or as a suppository.

Gelatin capsules may contain the therapeutic compound and powdered carriers such as lactose, sucrose, mannitol, starch, cellulose derivatives, magnesium stearate, stearic acid, and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a

5   period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration in the gastrointestional tract.

Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

10   In general, water, a suitable oil, saline, aqueous dextrose (glucose), and related sugar solutions and glycols are suitable carriers for parenteral solutions. Solutions for parenteral administration may contain a water soluble salt of the therapeutic compound (agent and the like), suitable stabilizing agents and, if necessary, buffer substances. Antioxidizing agents such as sodium bisulfate, sodium sulfite or ascorbic acid either alone or combined are suitable stabilizing agents.

15   Also used are citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives such as benzalkonium chloride, methyl- or propyl-prarben and chlorobutanol. Suitable pharmaceutical carriers are descried in *Remington's Pharmaceutical Sciences*, a standard reference text in this field.

Additionally, standard pharmaceutical methods can be employed to control the duration of

20   action. These are well known in the art and include control release preparations and can include appropriate macromolecules, for example polymers, polyesters, polyaminoacids, polyvinylpyrrolidone, ethylenevinylacetate, methyl cellulose, caraboxymethyl cellulose or protamine sulfate. The concentration of macromolecules as well as a the methods of incorporation can be adjusted in order to control release. Additionally, the agent can be incorporated into particles of

25   polymeric materials such as polyesters, polyaminoacids, hydrogels, poly (lactic acid) or ethylenevinylacetate copolymers. In addition to being incorporated, these agents can also be used to trap the compound in microcapsules.

Useful pharmaceutical dosage forms for administration of the compounds of this invention can be illustrated as follows.

Capsules: Capsules are prepared by filling standard two-piece hard gelatin capsulates each with 100 milligram of powdered active ingredient, 175 milligrams of lactose, 24 milligrams of talc and 6 milligrams magnesium stearate.

Soft Gelatin Capsules: A mixture of active ingredient in soybean oil is prepared and injected
5 by means of a positive displacement pump into gelatin to form soft gelatin capsules containing 100 milligrams of the active ingredient. The capsules are then washed and dried.

Tablets: Tablets are prepared by conventional procedures so that the dosage unit is 100 milligrams of active ingredient. 0.2 milligrams of colloidal silicon dioxide, 5 milligrams of magnesium stearate, 275 milligrams of microcrystalline cellulose, 11 milligrams of cornstarch and
10 98.8 milligrams of lactose. Appropriate coatings may be applied to increase palatability or to delay absorption.

Injectable: A parenteral composition suitable for administration by injection is prepared by stirring 1.5% by weight of active ingredients in 10% by volume propylene glycol and water. The solution is made isotonic with sodium chloride and sterilized.

15 Suspension: An aqueous suspension is prepared for oral administration so that each 5 millimeters contain 100 milligrams of finely divided active ingredient, 200 milligrams of sodium carboxymethyl cellulose, 5 milligrams of sodium benzoate, 1.0 grams of sorbitol solution U.S.P. and 0.025 millimeters of vanillin.

An embodiment of the present invention relates to a therapeutic compound comprising at
20 least one drug moiety, and at least one polypeptide drug carrier moiety, the drug moiety being covalently linked to the carrier moiety, and the polypeptide drug carrier moiety comprising glutamic acid and a second amino acid selected from the group consisting of aspartic acid, alanine, asparagine, glutamine, glycine, and combinations thereof. The drug moiety may be selected from the group consisting of anti-tumor drugs, anti-inflammatory drugs, drugs for the cardiovascular system,
25 diabetic drugs, metabolically-acting drugs, drugs for pain treatment and any other types of drugs where delivery via the inventive carrier is or may be desired. The drug moiety may be selected from the group of drugs consisting of paclitaxel, epipodophyllotoxin, vincristine, docetaxel, daunomycin, doxorubicin, mitoxantrone, topotecan, bleomycin, gemcitabine, fludarabine and 5-FUDR. In a preferred embodiment the drug moiety may be paclitaxel.

30 Conditions to be treated may include, but are in no way limited to, prostate, breast, ovarian, colon, leukemia, lymphoma, lung and liver cancers. For example and in a non-limiting sense,

paclitaxel may be conjugated with the inventive peptide and used to treat, for example, prostate, breast, ovarian, colon, leukemia, lymphoma, lung and liver cancers.

Further, in a specific embodiment, the polypeptide drug carrier moiety comprises from about 50 to about 90 percent, by weight, glutamic acid, and from about 10 to about 50 percent, by weight,

5   aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof, more preferably from about 10 to about 60 percent, by weight, glutamic acid, and from about 20 to about 50 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof, and most preferably from about 20 to about 40 percent, by weight, glutamic acid, and from about 25 to about 30 percent, by weight, aspartic acid, or alanine, or asparagine, or

10   glutamine, or glycine, or combinations thereof.

In an embodiment, the therapeutic compound may comprise at least two drug moieties, which may not the same as each other, and/or the therapeutic compound may comprise a plurality of drug moieties.

In still another embodiment, the drug moiety of the therapeutic compound comprises from

15   about 10 percent to about 60 percent, by weight, more preferably from about 20 percent to about 50 percent, by weight, and most preferably from about 20 percent to about 40 percent, by weight of the therapeutic compound. Further, the polypeptide drug carrier moiety may comprise from about 40 percent to about 90 percent, by weight, more preferably from about 50 percent to about 80 percent, by weight, and most preferably from about 60 percent to about 80 percent, by weight of the

20   therapeutic compound.

In a preferred embodiment, the drug moiety may be paclitaxel, the carrier moiety may comprise about 70 percent glutamic acid and about 30 percent aspartic acid, the paclitaxel drug moiety may be about 20 percent to about 40 percent, by weight, of the therapeutic compound, and the molecular weight of the compound may be from about 20,000 to about 50,000 daltons.

25   Another aspect of the present invention relates to a method for improving the solubility of a drug moiety comprising the steps of covalently conjugating at least one drug moiety with at least one polypeptide drug carrier moiety comprising glutamic acid and a second amino acid selected from the group consisting of aspartic acid, alanine, asparagine, glutamine, and glycine, and combinations thereof, thereby creating a therapeutic compound, the therapeutic compound comprising at least one

30   drug moiety, and at least one polypeptide drug carrier moiety, the drug moiety being covalently linked to the carrier moiety, and the polypeptide drug carrier moiety comprising glutamic acid and

aspartic acid or alanine, or asparagine, of glutamine, or glycine, or combinations thereof. In a preferred embodiment, the water solubility of the therapeutic compound is greater than the water solubility of the drug moiety. In another embodiment, the drug moiety may be an antitumor drug. In a preferred embodiment, the drug moiety may be paclitaxel.

5   Further, in a specific embodiment, the polypeptide drug carrier moiety may comprise from about 50 to about 90 percent, by weight, glutamic acid, more preferably from about 60 to about 80 percent, by weight, glutamic acid, and most preferably from about 70 to about 75 percent, by weight, glutamic acid, and from about 10 to about 50 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof, more preferably from about 20 to

10   about 40 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof, and most preferably from about 25 to about 30 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof.

Still another aspect of the present invention relates to a method for treating a condition comprising the steps of administering a therapeutically effective amount of a therapeutic compound

15   comprising at least one drug moiety, and at least one polypeptide drug carrier moiety, the drug moiety being covalently linked to the carrier moiety, and the polypeptide drug carrier moiety comprising glutamic acid and a second amino acid selected from the group consisting of aspartic acid, alanine, asparagine, glutamine, and glycine, and combinations thereof. In a specific embodiment, the drug moiety may be selected from the group consisting of anti-tumor drugs, anti-

20   inflammatory drugs, drugs for the cardiovascular system, diabetic drugs, metabolically-acting drugs, drugs for pain treatment and any other types of drugs where delivery via the inventive carrier is or may be desired. The drug moiety may also be selected from the group of drugs consisting of paclitaxel, epipodophyllotoxin, vincristine, docetaxel, daunomycin, doxorubicin, mitoxantrone, topotecan, bleomycin, gemcitabine, fludarabine and 5-FUDR. In an embodiment, the polypeptide

25   drug carrier moiety may comprise from about 50 to about 90 percent, by weight, glutamic acid, more preferably from about 60 to about 80 percent, by weight, glutamic acid, and most preferably from about 70 to about 75 percent, by weight, glutamic acid, and from about 10 to about 50 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or combinations thereof, more preferably from about 20 to about 40 percent, by weight, aspartic acid, or alanine, or

30   asparagine, or glutamine, or glycine, or combinations thereof, and most preferably from about 25 to about 30 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or

combinations thereof. In a preferred embodiment, the condition may be a prostate tumor and the drug moiety may be paclitaxel.

Further, a key aspect of this invention is the discovery that a particular polypeptide comprising a di-peptide monomer repeating unit of glutamic acid and aspartic acid when covalently
5   conjugated to drugs imparts enhanced solubility upon drugs as well as enhanced and/or unique biological properties. This discovery is exemplified in detail below in one preferred embodiment, the conjugation of paclitaxel to a polyglutamic acid-aspartic acid polypeptide and its use in, for example, treatment of prostate cancer. This example is merely illustrative, however, of the broader scope of the invention which encompasses the conjugation of any drug to the inventive polymer
10   comprising a poly(di-peptide) polymer composed of glutamic acid and aspartic acid, or glutamic acid and alanine, or glutamic acid and asparagine, or glutamic acid and glutamine, or glutamic acid glycine, or glutamic acid and combinations of one or more amino acids selected from the group of alanine, asparagine, glutamine, and glycine.

In general, then, it is understood that the present invention is not limited to the conjugation
15   of any particular drug but rather, encompasses the conjugation of a wide range of drugs, both known and presently unknown, readily water soluble or poorly water soluble and of varying biological effects. This includes, for example, antitumor drugs, and other drugs such as anti-inflammatory drugs, drugs for the cardiovascular system, diabetic drugs, metabolically-acting drugs, drugs for pain treatment and any other types of drugs where delivery via the inventive carrier is or may be desired.
20   Specific, but non-limiting examples of drugs include but are not limited to paclitaxel, epipodophyllotoxin, vincristine, docetaxel, daunomycin, doxorubicin, mitoxantrone, topotecan, bleomycin, gemcitabine, fludarabine and 5-FUDR.

Furthermore, it is not necessarily contemplated that the present invention be limited to strictly the use of a polypeptide containing repeating monomers comprised of aspartic acid and
25   glutamic acid, or alanine and glutamic acid, or asparagine and glutamic acid, or glutamine and glutamic acid, or glycine and glutamic acid. While at this time, a preferred embodiment is a polymer consisting of glutamic acid and aspartic acid (and indeed details of the preferred embodiment are provided in the illustrative examples below), it is not contemplated that this be a limited example of the full scope of the invention. For example, it is contemplated that the inventive polymer drug
30   carrier need not be exclusively composed of polyglutamic acid/aspartic acid (or alanine, or asparagine, or glutamine, or glycine) either as repeating monomer polypeptides or in mixed

combinations. Indeed the noted amino acids (for example, glutamic acid and aspartic acid (or alanine, or asparagine, or glutamine, or glycine)) may make up some percentage of the overall polypeptide carrier. Further, the carrier may comprise other components than the noted amino acids, providing that at least some of the carrier is composed of the inventive polypeptide combination.

5   Furthermore, the invention is not restricted solely to use of "wild type" amino acids in the polymer. Rather, the invention encompasses any number of changes to the structure of these amino acids which would result in polypeptides having essentially the same function and/or structure. The amino acids may be D amino acids, L amino acids or mixtures of D and L amino acids. Further still, it is contemplated that the drug conjugate peptide of the present invention need not exclusively

10   contain an individual polypeptides containing 100% glutamic/aspartic acid (or alanine, or asparagine, or glutamine, or glycine). Rather, while sections of the polypeptide may contain the noted amino acids, it is believed that it is not necessary for the entire peptide to homogeneously include the only the noted amino acids, especially not necessarily in repeating monomers.

What is important is that the inventive peptide contain at least glutamic acid in the noted

15   proportions (see above, for example, from 50 to about 90 percent, by weight, glutamic acid, more preferably from about 60 to about 80 percent, by weight, glutamic acid, and most preferably from about 70 to about 75 percent, by weight, and at least aspartic acid, or alanine, or asparagine, or glutamine, or glycine, or any combination thereof including combinations with aspartic acid, in the noted proportions (see above, for example, from about 10 to about 50 percent, by weight, aspartic

20   acid, or alanine, or asparagine, or glutamine, or glycine, more preferably from about 20 to about 40 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine, and most preferably from about 25 to about 30 percent, by weight, aspartic acid, or alanine, or asparagine, or glutamine, or glycine. The inventive polypeptide is further envisioned to preferably have a molecular weight ranging from about 20,000 to about 50,000 daltons.

25   The following detailed examples of the preferred embodiment relate to an illustrative example of the present invention wherein the poorly soluble antitumor drug paclitaxel is conjugated to the inventive di(glutamate/aspartate) polypeptide drug carrier and the resulting product is shown to have unique and indeed surprising biological activity, for example, against prostate cancer *in vivo*. Also disclosed are methods for producing the inventive polypeptide drug carrier and sample

30   conjugates. It is to be understood that these examples are in no way intended to limit the scope of

the present invention but merely illustrate one example of a preferred embodiment presently known to the inventors. Additional, embodiments are within the scope of the present invention.

Example 1
5   Synthesis of poly(dipeptide) polypeptide

The following sections report on preferred, but not limiting, embodiments for synthesizing the inventive polyglutamic acid/aspartic acid (or poly glutamic acid/alanine, or poly glutamic acid/asparagine, or poly glutamic acid/glutamine, or poly glutamic acid/glycine) copeptide and properties of such a peptide. In general, the inventive poly(glutamic/aspartic acid) di-peptide is a
10 biodegradable polymer. As described below, the polypeptide may be synthesized in a conjugate form with a particular drug in order to enhance the solubility and/or *in vivo* deliverability of such drug. In such an instance, it may be considered as a "propolymeric drug delivery vehicle" and be prepared in a powder form. By adding sterile to the powder, the drug conjugate can then be used for interveneous administration. The inventive polymer-drug conjugates provide sustained relief
15 properties and prolong blood circulation time which are more effective and less toxic than using, for example, unconjugated drug alone. As discussed above, examples of drugs which can be conjugated to the inventive conjugating include, in a non-limiting sense, paclitaxel, epipodophyllotoxin, vincristine, docetaxel, daunomycin, doxorubicin, mitoxantrone, topotecan, bleomycin, gemcitabine, fludarabine and 5-FUDR

20 In one embodiment, for example, the inventive poly(glutamate/aspartate) polypeptide is approximately 26,000 to 30,000 dalton molecular weight containing approximately 70% glutamic acid and 30% aspartic acid. Hence, it can be seen that the inventive copolymer need not necessarily contain a homogeneous and repeating di-peptide, which would result in a 50-50 content of glutamic acid and aspartic acid. Rather, many variations within this range are contemplated. For example,
25 the preferred embodiment may contain 70% glutamic acid and 30% aspartic acid. However, this range could extend from 50-90% (weight) glutamic acid and 10-50% (weight) aspartic acid.

Example 2
Experimental Procedure-Synthesis of Poly(glutamate/aspartate) Polypeptides

30 Using a known procedure, N-carboxyanhydride (NCAs) was prepared by phosgenation of the corresponding β–benzyl-l-aspartate and γ-benzyl-l-glutamate (Idelson, M., Blout, E.R., *J. Am.*

Chem. Soc. **1958**, 80, 2387-2393; Karlson, R.H., Norland, K.S., Fasman, G.D., Blout, E.R., *J. Am. Chem. Soc.* **1960**, 82, 2268-2278; Paolillo, L., Temussi, P.A., Bradbury, E.M., Crane-Robinson, C. *Biopolymers,* **1972**, 11, 2043-2052; Hayashi, T., Iwatsuki, M., *Biopolymers,* **1990**, 29, 549-557; Bradbury, E.M., Carpenter, B.G., Crane-Robinson, C., Goldman, H., *Macromolecules,* **1971**, 4, 557-

5   564.). Briefly, a solution of phosgene (10% w/v) was bubbled into ethylacetate (150 ml). An aliquot (10 ml) of this solution was added to 10 grams of finely ground β–benzyl-l-aspartate and γ–benzyl-l-glutamate in ethylacetate (150 ml). The reaction was stirred under reflux for 5 min. A stream of nitrogen was employed to remove excess HCl prior to the next addition of phosgene. The sequence was repeated until no traces of suspended amino acid HCl remained. The mixture was

10   then filtered and the solvent was evaporated under Vacuo. The product was crystallized from ethyl acetate.

Solutions of NCAs of β–benzyl-1-aspartate and γ–benzyl-1-glutamate in dioxane/methylene chloride (1:3) were prepared. The ratios (w/w) used between δ-benzyl-l-aspartate and γ-benzyl-l-glutamate were 3:7, 2:8 and 1:9. The polymerization was initiated with triethylamine in methylene

15   chloride (4 ml, 2.5% v/v). The copolymerization reaction was under reflux for 30 min and followed by CO_2 evolution. The reaction was stopped at about 30 mol % conversion. The polymers formed were precipitated by adding ice cold methanol containing 0.IN HCl (5%) v/v). The products were washed with methanol and dried under reduced pressure, yielded 8 gm (for 3:7 batch). The debenzylation was conducted by using HBr according to a known procedure (Idelson, M.; Blout,

20   E.R., *J. Am. Chem. Soc.* **1958**, 80, 2387-2393). After HBr treatment, the aqueous solution was dialyzed against distilled water, filtered through Millipore filter and lyophilized. Typical average molecular weight was 26,000-30,000 daltons. A synthetic scheme is shown in Figure 1A. A similar technique was used to prepare polymers of glutamic acid and alanine, glutamic acid and asparagine, glutamic acid and glutamine, glutamic acid and glycine, and glutamic acid and one or more amino

25   acids from the group consisting of aspartic acid, alanine, asparagine, glutamine, and glycine.

Amino acid analyzer (PE/ABI 42OA) (Foster City, CA) was used to determine the actual composition ratio of aspartic acid and glutamic acid. Briefly, poly(dipeptide) was hydrolyzed with HCl (6N) at 150°C for 75 min. The hydrolyzed products were loaded on PVDF membrane and methanol (30%) and HCl (0.1N, 0.2 ml) were added to extract the amino acids. Using pre-column

30   derivatization with phenylisothiocyanate, the amino acid concentration was determined. An amino acid analysis of the poly(glutamic acid/aspartic acid) is shown in Figure 1B.

Example 3

Anti-Cancer Drug Delivery Using Poly(glutamate/aspartate) Polypeptide as a Drug Carrier

In order to demonstrate one embodiment of the present invention, a conjugate of the antitumor agent paclitaxel was made with the inventive polypeptide and used as a drug delivery

5   vehicle. It was then shown that this inventive conjugate possessed superior biological and therapeutic properties *in vivo* over, for example, unconjugated drug, and the drug conjugated with known prior art carriers (*e.g.*, homopolymers of glutamic acid and aspartic acid). Data below shows that, for example, conjugating the antitumor drug paclitaxel to the inventive polymer–and only the inventive polymer– results in unexpected therapeutic properties of paclitaxel such as the treatment

10   of prostate cancer. Indeed, the following results show what applicants believe is the first described efficacy of paclitaxel in any form against prostate tumors *in vivo*.

Example 4

Experimental Procedure-Synthesis of Poly(glutamate/aspartate) - Paclitaxel Conjugates

15   Numerous studies have suggested that limited polymer-drug conjugate discretion through the kidneys is evident when the molecular weight of the conjugate ranges from 20,000 to 50,000 daltons. Thus, to enhance tumor uptake of the paclitaxel-inventive carrier conjugate, a molecular weight range of a conjugate of 26,000 to 30,000 daltons was selected.

Conjugation of paclitaxel to poly(glutamate/aspartate) was conducted by using drug:polymer

20   molar ratio of 1:4 in N,N-dimethylformaide (DMF). Dicyclocarbodiimide (DCC) was used as a coupling agent. In a typical run, poly(dipeptide) (383 mg) was dissolved in DMF (8 ml) and DCC (152.2 mg) was added. To this mixture, N,N-dimethylaminopyridine (8.5 mg) and paclitaxel (209.4 mg) were added. The reaction was stirred for 22 hours under room temperature. The urea was filtered and the resulting solvent was added to chloroform. The product was filtered and redissolved

25   in sodium bicarbonate (IN). After dialysis (cut off at 10,000) against distilled water, the product was freeze-dried and weighed; 620 mg. The product contained 26.79% paclitaxel. Nuclear magnetic resonance (NMR) spectra was recorded on a GE GN-500 Spectrometer. A synthetic scheme of conjugating paclitaxel to poly(glutamate/aspartate) is shown in Figure 2A.

Proton nuclear magnetic resonance ('HNMR) of poly(dipeptide), paclitaxel and water soluble

30   poly(dipeptide)-paclitaxel conjugates were conducted using GE 600MHz NMR. The spectrums are shown in Figures 2B, 2C and 2D. In the paclitaxel conjugates, apparently, C2' position of paclitaxel

was linked to the conjugates. For instance, the chemical shift (δ) of C2' was 4.68 (doublet (d) in paclitaxel (Figure 2B) and 4.91(d) in the paclitaxel conjugates (Figure 2D).

Example 5

5   Determination of paclitaxel concentration in poly(glutamate/aspartate) paclitaxel conjugates:

To compare the difference between paclitaxel and its conjugates in ultra violet (UV) absorption, UV scans (Beckman DU-640 spectrometry, Fullerton, CA) of these derivatives were recorded (Figures 3A, 3B, 4A and 4B). Absorbency of paclitaxel at various concentrations in methanol (2,6,14 and 18 µg/ml) was determined by UV at 232 nm. A standard curve was then

10 generated (Figure 5A). Polymer paclitaxel conjugates was dissolved in water and absorbency of an aliquot of this solution was determined. Paclitaxel concentration in the conjugates was determined by extrapolating to the standard curve (Figure 5B).

Example 6

15 Chromatographic analysis of poly(glutamate/aspartate) paclitaxel conjugates:

To demonstrate the purity of poly(glutamate/aspartate) paclitaxel conjugates, high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) were used. For HPLC, a Nova-Pak C-18 reverse phase column (3.9x15 mm) was used (Figures 6, 7, 8, 9A, 9B and 9C). The compounds were under the same concentration and eluted with methanol/water (2:1) at

20 the flow rate of a ml/min. For TLC, a silica gel-coated plate was used. The product was eluted with chloroform/methanol (7:3).

Example 7

Solubility and stability of poly(glutamate/aspartate) paclitaxel conjugates:

25 Solubility of the conjugates was determined in saline (0.9%) at 25°C. Stability assay was conducted in phosphate buffered saline (pH 7.4) at 25°C. An aliquot of sample at various time was assayed by HPLC (Figures 9D and 9E).

Example 8

30 In vitro cell culture assay:

To evaluate cytotoxicity of paclitaxel and the conjugates against mammary tumor cells, three human tumor cell lines were selected: PC3 (prostate); KB (nasopharyngeal); and, MDA MB 231 (breast). All cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2 in Eagle's medium. Forty-eight hours prior to the experiment, the cells were transferred to 35 mm culture

5   dishes at 5 x 10^5 cells per dish and grown to 80% confluence. Cultured human tumor cells in 35 mm dishes were incubated with either paclitaxel or conjugates at various concentrations. The incubation was stopped at 72 hours. Methylene tetrazolium (MTT) dye assay determined the amount of viable cells. Cellular protein content was determined by Lowry assay. The drug concentration that inhibits 50% of cell growth was then determined. At higher concentrations (e.g., 1 micro molar) there was

10   no difference in cell inhibition between paclitaxel and the conjugates. However, cell inhibition was more pronounced in the paclitaxel group at lower concentrations (Figure 9F).

Example 9

Evaluation of the conjugates in four tumor-bearing animal models:

15   Paclitaxel is known to produce an anticancer effect against breast and ovarian tumors, and not in the treatment of prostate cancer. Therefore, four animal models were selected: ovarian, breast and two prostate cancer models. The ovarian animal model was driven from animal tumor cell line, the other three models were created using human cell lines xenografted in nude mice.

20   Example 10

Ovarian tumor-bearing animal model:

Female C3H/Kam mice (20-28 g, n=5/dose) were inoculated with ovarian tumor cells (OCA-1,500,000/mouse, subcutaneously (s.c.)) in the hind leg. When the tumor reached 500 mm³, the mice were administered either conjugates or paclitaxel doses of 40-160 mg/kg (conjugates) or 80 mg/kg

25   (paclitaxel). For comparison purposes, a parallel study was conducted comparing our product with other water soluble paclitaxel products; mice were administered poly(glutamic acid) paclitaxel conjugates at doses of 40-160 mg/kg. Tumor volumes and body weight were recorded daily for sixty days. Tumor volumes were measured: [length (1) x width (w) x thickness (h)]/2. Loss of body weight of 15% is considered a chemical-induced toxic effect. These results are shown in Figures

30   10 and 11 and indicate that while conjugation of paclitaxel to a polypeptide enhances its anti-ovarian tumor efficacy substantially, there is no apparent difference between paclitaxel in the inventive

conjugate compared with paclitaxel conjugataed to a prior art homopolymer carried polyglutamic acid.

Example 11

5   Breast tumor-bearing animal model:

Athymic female nude mice (NCr5-nu/mu) were inoculated with human breast cancer cells (MDA435, 10^6 cells/mouse, n=5/dose s.c. in the mouse mammary fat pad. After 15-20 days and a tumor volume of 250 mm^3 , the mice-bearing human breast tumor were administered either the conjugates or paclitaxel at doses of 60-100 mg/kg (conjugates) or 60 mg/kg (paclitaxel). Tumor

10 volumes and body weight were recorded daily for sixty days. Tumor volumes were measured as [length (1) x width (w) x thickness (h)]/2. Loss of body weight of 15% is considering a chemical-induced toxic effect. The results are shown in Figure 12 and indicate that unconjugated paclitaxel, paclitaxel conjugated to a prior art cancer polyglutamic acid humopolymer and the inventive conjugate are all effective *in vivo* against human breast cancer.

15

Example 12

Prostate tumor-bearing animal models:

Athymic male nude mice (NCr-nu/nu) were inoculated with two types of human prostate cancer cells (A10 and PC3, 10^6 cells/mouse, n=5/dose) s.c. in the mouse mammary fat pad. Both

20 cell lines were obtained from the Department of GU oncology at The University of Texas M.D. Anderson Cancer Center. A10 cell line expresses PSA and androgen receptors (22). PC3 cell line does not overexpress PSA and androgen receptors. After 15-20 days with tumor volume 500 mm^3, the mice-bearing human prostate tumors were administered either the conjugates or paclitaxel at the doses 60-120 mg/kg (conjugates) or 60 mg/kg (paclitaxel). Figure 13A shows that paclitaxel

25 conjugated with polyglytamic acid/aspartic acid is effective against prostate cancer *in vivo* whereas unconjugated paclitaxel is not effective.

For comparison purposes, a parallel study was conducted comparing the inventive paclitaxel conjugate with other water-soluble paclitaxel products, including paclitaxel conjugated to the prior art polymer polyglutamic acid. Mice were administered with poly(glutamic acid) paclitaxel

30 conjugates at the dose 120 mg/kg. Poly(glutamic acid) paclitaxel conjugates were prepared using a known procedure (Li, C.; Yu, D-F.; Newman, R.S.; Cabral, F.; Stephens L.C.; Hunger, N.; Milas,

L.; Wallace, S. *Cancer Res.* 1998, 58, 2404-2409). Tumor volumes and body weight were recorded

daily up to sixty days. Tumor volumes were measured as [length (1) x width (w) x thickness (h)]/2.

Loss of body weight of 15% is considered a chemical-induced toxic effect. Figure 13B shows that

paclitaxel conjugated to polyglutamic polypeptide is ineffective against prostate cancer *in vivo*.

5

Example 13

Histopathology of tumor tissue after treatment:

After treatment with either paclitaxel or the polymer conjugates, tumor tissues (breast and

prostate) were dissected and embedded in formalin. The tumor tissue was fixed in paraffin and

10   stained with eosin or hematoxycilin. Apoptosis process produced by paclitaxel or the polymer

conjugates was recorded by microscopic observation.

Example 14

15   Synthesis of poly(glutamate/aspartate) conjugates of paclitaxel:

Poly(glutamate/aspartate) at 26,000-30,000 molecular weight range was prepared. The

poly(dipeptide) contains 70% glutamic acid and 30% aspartic acid as determined from amino acid

analyzer (Figure 1C). Conjugation of paclitaxel to poly(glutamate/aspartate) was conducted by

using drug:polymer molar ratio of 1:4 in N,N-dimethylformaide. UV scans of paclitaxel, the

20   conjugates and poly(dipeptide) are shown in Figures 3 and 4. The standard curve is shown in Figure

5. Typical paclitaxel concentration in conjugates ranged from 20-40%.

Example 15

Chromatographic analysis of poly(glutamate/aspartate) paclitaxel conjugates:

25   HPLC analysis of the product showed that retention times for paclitaxel, the conjugates and

the polymer of 4.2 min, 1.0 min and 1.0 min as shown in Figures 6-8. Though no difference

between the conjugates and the polymer was reflected, the absorbency of the conjugates was

significantly higher than the polymer alone. When mixed, the known amount of paclitaxel, a

different retention time was noted (shown in Figures 9A, 9B and 9C). For TLC, the retarded factor

30   (Rf) for paclitaxel and the conjugates was 0.8 and 0.1.

Example 16

Solubility and stability of poly(glutamate/aspartate) paclitaxel conjugates:

Solubility of conjugates was determined to be 20 mg/ml in saline, which is almost 3,000 times better than paclitaxel. Stability assay showed the useful half-life of the conjugates was 18 days

5   in phosphate buffered saline (pH 7.4) at 25°C (Figures 9D and 9E).

Example 17

In vitro cell culture assay:

The cytotoxicity (IC-50) of paclitaxel is about twenty times more potent than the conjugates

10   in the PC3 cell line tested (Figure 9G). This difference may be due to sustained release of paclitaxel from the conjugates.

Example 18

In vivo antitumor activity studies:

15   Poly(glutamate/aspartate) paclitaxel conjugates produced better antitumor effects than paclitaxel in all four animal models tested (shown in Figures 10, 12-14). When compared to poly(glutamic acid) paclitaxel conjugates, ply(glutamate/aspartate) paclitaxel conjugates had equivalent anti-tumor effects (Figures 10 and 11). However, poly(glutamate/aspartate) paclitaxel conjugates proved to be more potent than either poly(glutamic acid) paclitaxel or paclitaxel alone

20   in prostate cancer animal models (shown in Figures 13A, 13B and 14). Despite the fact the higher initial loading dose could be provided by poly(glutamate/aspartate) paclitaxel conjugates, moreover, poly(glutamate/aspartate) paclitaxel conjugates did not alter body weight loss suggesting it is less toxic than paclitaxel. In Figures 15, 15B, 16, 1 7, 17B and 17C, tumor shrinkage in human tumor-bearing animal models for poly(glutamate/aspartate) paclitaxel conjugates is more pronounced than

25   either poly(glutamic acid) paclitaxel or paclitaxel alone.

Example 19

Histopathology of tumor tissue after treatment:

After treatment with either paclitaxel or the polymer conjugates, apoptosis process produced

30   by the polymer conjugates was more pronounced than paclitaxel using microscopic observation.

In summary, a new poly(dipeptide) based water soluble paclitaxel is developed. The solubility is increased up to 20 mg/ml. The half-life of *in vitro* stability in phosphate buffered saline (pH 7.4) is 18 days. The product is easily scaled up and prepared as a sterilized powder. Compared to paclitaxel, insignificant toxicity was observed and much higher initial loading does could be administered intravenously. The product produced significant anticancer effects in ovarian, breast and prostate cancer models. In human prostate tumor-bearing nude mice, the product is effective as opposed to poly(glutamic acid) paclitaxel conjugates and unconjugated paclitaxel which did not show therapeutic effect against prostate tumors.

All patents and publications mentioned in this specification are indicative of levels of those skilled in the art to which the invention pertains. All patents and publications are herein incorporated by reference to the same extent as if each individual publication is specifically and individually indicated to be incorporated by reference.

One skilled in the art will readily appreciate the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods, procedures, treatments, molecules and specific compounds described herein are presently representative of preferred embodiments, are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention and are defined by the scope of the claims.

CLAIMS

What is claimed is:

1   1.   A therapeutic compound comprising:

2   at least one drug moiety, and

3   at least one polypeptide drug carrier moiety having from about 50% to about 90% by weight,

4   glutamic acid, and from about 10% to about 50%, by weight, of a second amino acid selected from

5   the group consisting of aspartic acid, alanine, asparagine, glutamine, and glycine,

6   the drug moiety being covalently linked to the carrier moiety.

1   2.   The therapeutic compound of claim 1, wherein the drug carrier moiety has a

2   molecular weight from about 20,000 daltons to about 50,000 daltons.

1   3.   The therapeutic compound of claim 1, wherein the second amino acid is aspartic acid.

1   4.   The therapeutic compound of claim 1, wherein the second amino acid consists of two

2   or more amino acids selected from the group consisting of aspartic acid, alanine, asparagine,

3   glutamine, and glycine.

1   5.   The therapeutic compound of claim 1, wherein the drug moiety is an anti-tumor drug.

1   6.   The therapeutic compound of claim 1, wherein the drug moiety is selected from the

2   group consisting of paclitaxel, epipodophyllotoxin, vincristine, docetaxel, daunomycin, doxorubicin,

3   mitoxantrone, topotecan, bleomycin, gemcitabine, fludarabine and 5-FUDR.

1   7.   The therapeutic compound of claim 1, wherein the drug moiety is paclitaxel.

1   8.   The therapeutic compound of claim 1, wherein the polypeptide drug carrier moiety

2   comprises from about 60% to about 80%, by weight, glutamic acid, and from about 20% to about

3   40%, by weight, of the second amino acid:

1   9.   The therapeutic compound of claim 8, wherein the second amino acid is aspartic acid.

1   10.   The therapeutic compound of claim 8, wherein the second amino acid consists of two
2   or more amino acids selected from the group consisting of aspartic acid, alanine, asparagine,
3   glutamine, and glycine.

1   11.   The therapeutic compound of claim 1, wherein the polypeptide drug carrier moiety
2   comprises from about 70% to about 75%, by weight, glutamic acid, and from about 25% to about
3   30%, by weight, of the second amino acid.

1   12.   The therapeutic compound of claim 11, wherein the second amino acid is aspartic
2   acid.

1   13.   The therapeutic compound of claim 11, wherein the second amino acid consists of
2   two or more amino acids selected from the group consisting of aspartic acid, alanine, asparagine,
3   glutamine, and glycine.

1   14.   The therapeutic compound of claim 1, comprising at least two different drug moieties.

1   15.   The therapeutic compound of claim 1, comprising a plurality of drug moieties.

1   16.   The therapeutic compound of claim 1, wherein the drug moiety comprises from about
2   10 percent to about 60 percent, by weight, of the therapeutic compound.

1   17.   The therapeutic compound of claim 1, wherein the polypeptide drug carrier moiety
2   comprises from about 40 percent to about 90 percent, by weight, of the therapeutic compound.

1   18.   The therapeutic compound of claim 1, wherein the drug moiety comprises from about
2   20 percent to about 50 percent, by weight, of the therapeutic compound.

1   19.   The therapeutic compound of claim 1, wherein the drug moiety comprises from about
2   20 percent to about 40 percent, of the therapeutic compound.

1   20.   The therapeutic compound of claim 1, wherein the amino acids can be in L form, or

2   D form, or a racemic mixture of L and D forms.

1   21.   The therapeutic compound of claim 1, wherein

2   the drug moiety is paclitaxel and is about 24 percent to about 30 percent, by weight, of the

3   therapeutic compound,

4   the carrier moiety comprises about 70 percent glutamic acid and about 30 percent aspartic

5   acid, and

6   the molecular weight of the therapeutic compound is from about 26,000 to about 30,000

7   daltons.

1   22.   A method for improving the solubility of a drug moiety comprising the steps of:

2   covalently conjugating at least one drug moiety with at least one polypeptide drug carrier

3   moiety, thereby creating a therapeutic compound, the therapeutic compound comprising:

4   at least one drug moiety, and

5   at least one polypeptide drug carrier moiety having about 50% to about 90%, by weight,

6   glutamic acid and about 10% to about 50% by weight, of a second amino acid selected from the

7   group consisting of aspartic acid, alanine, asparagine, glutamine, and glycine,

8   the drug moiety being covalently linked to the carrier moiety.

1   23.   The method of claim 22, wherein the drug carrier moiety has a molecular weight from

2   about 20,000 daltons to about 50,000 daltons.

1   24.   The method of claim 22, wherein the second amino acid is aspartic acid.

1   25.   The method of claim 22, wherein the second amino acid consists of two or more

2   amino acids selected from the group consisting of aspartic acid, alanine, asparagine, glutamine, and

3   glycine.

1   26.   The method of claim 22, wherein the water solubility of the therapeutic compound

2   is greater than the water solubility of the drug moiety.

1   27.   The method of claim 22, wherein the drug moiety is an antitumor drug.

1   28.   The method of claim 22, wherein the drug moiety is paclitaxel.

1   29.   The method of claim 22, wherein the polypeptide drug carrier moiety comprises from

2   about 60 to about 80 percent, by weight, glutamic acid, and from about 20 to about 40 percent, by

3   weight, of the second amino acid.

1   30.   A method for treating a condition comprising the steps of:

2   administering a therapeutically effective amount of a therapeutic compound comprising:

3   at least one drug moiety, and

4   at least one polypeptide drug carrier moiety having about 50% to about 90% by weight,

5   glutamic acid, and from about 10% to about 50%, by weight, of a second amino acid selected from

6   the group consisting of aspartic acid, alanine, asparagine, glutamine, and glycine,

7   the drug moiety being covalently linked to the carrier moiety.

1   31.   The method of claim 30, wherein the drug carrier moiety has a molecular weight from

2   about 20,000 daltons to about 50,000 daltons.

1   32.   The method of claim 30, wherein the second amino acid is aspartic acid.

1   33.   The method of claim 30, wherein the second amino acid consists of two or more

2   amino acids selected from the group consisting of aspartic acid, alanine, asparagine, glutamine, and

3   glycine.

1   34.   The method of claim 30, wherein the drug moiety is an anti-tumor drug.

1   35.   The method of claim 30, wherein the drug moiety is paclitaxel and the condition is

2   selected from the group consisting of prostate, breast, ovarian, colon, leukemia, lymphoma, lung and

3   liver cancers.

1   36.   The method of claim 30, wherein the condition is a prostate tumor and the drug

2   moiety is paclitaxel.
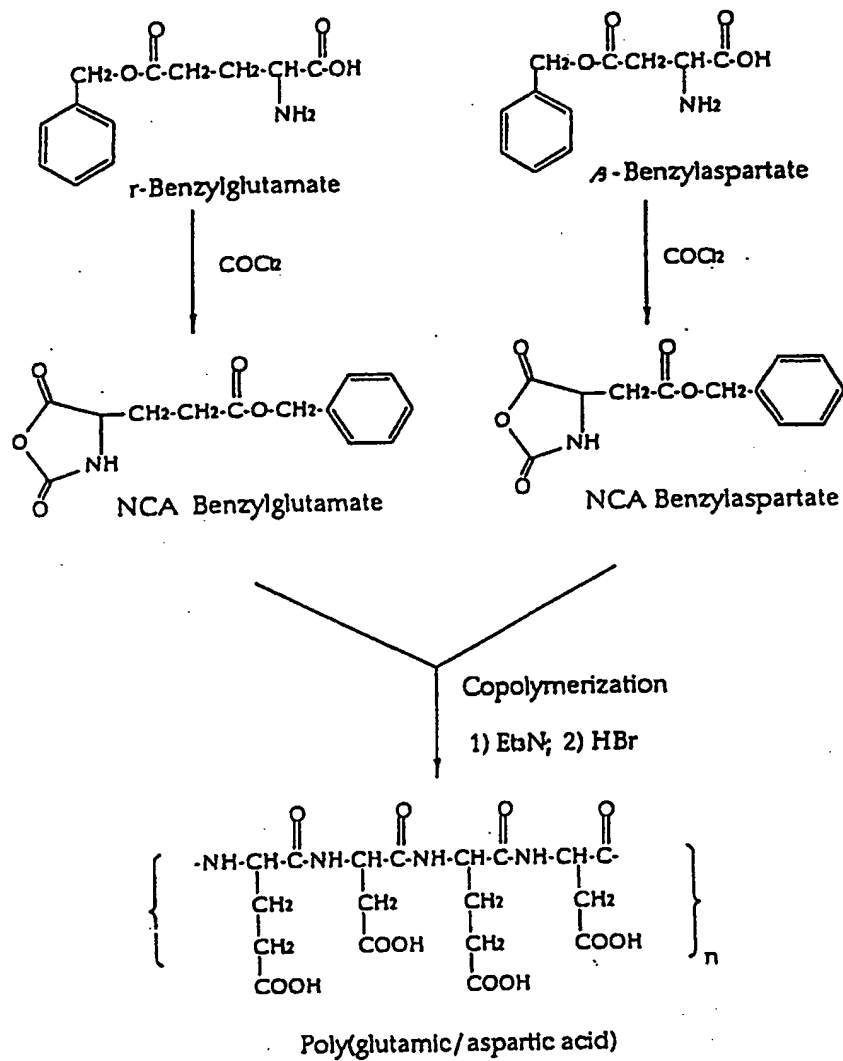
Figure 1A.

Synthesis of Poly(glutamic/aspartic acid)

Mol Percent Report

Sample ID: 01402017   (initiated 1/15/99 6:14am) BASELINE CORRECTED

Turntable Position: 6 8   Sampling Interval: 1.0 sec
Data Start   :   3.00 min   Samples In Run   : 72
Data Duration   :   16.00 min   Operator ID   :
Peak Ht Threshold :   3000 uAU   Int. Std. Amt   : 250 pmol

Calibration File :   14JANCAL   (initiated 1/15/99 9:44am)
Reference Time   :   0.00 min   (No ISTD Peak Specified)
Reference Offset 1:   0.03 min
Reference Offset 2:   0.00 min

Integration Interval: 0.0 to 16.0 min

        PEAK   RET.   PMOL   PMOL
         ID   TIME   BY   correc.   MOL
                    min   HEIGHT   INT STD   %
ASX   2.22   1054.53   0.00   30.74 (aspartic acid)
GLX   2.65   2375.83   0.00   69.26 (glutamic acid)

TOTAL PMOLS RECOVERED   3430.36

Minimum Peak Threshold: 3000 uAU   (55 peaks below threshold)
                                      (9 peaks found   )
                                      (2 peaks matched   )

Figure 1B.

Amino Acid Analysis of the Poly(glutamic acid/aspartic acid)
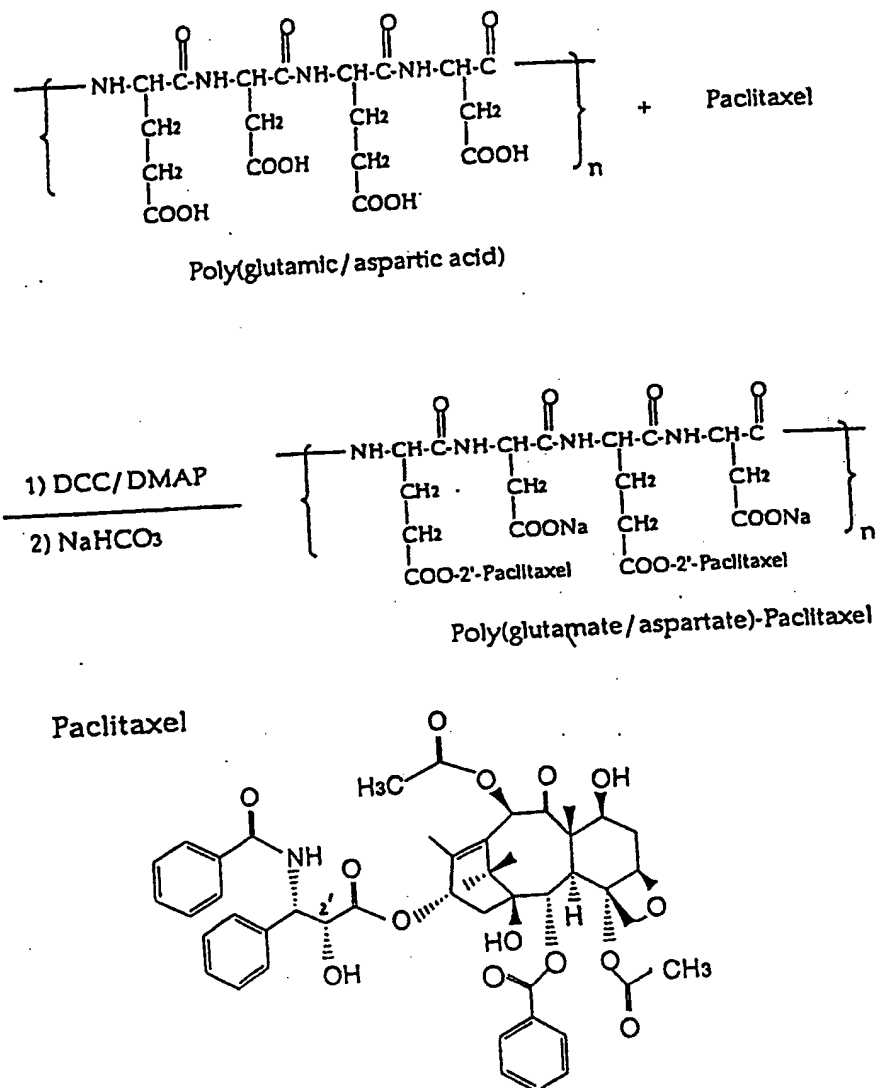
Figure 2A.

Synthesis of Paclitaxel to Poly(glutamic/aspartic acid)
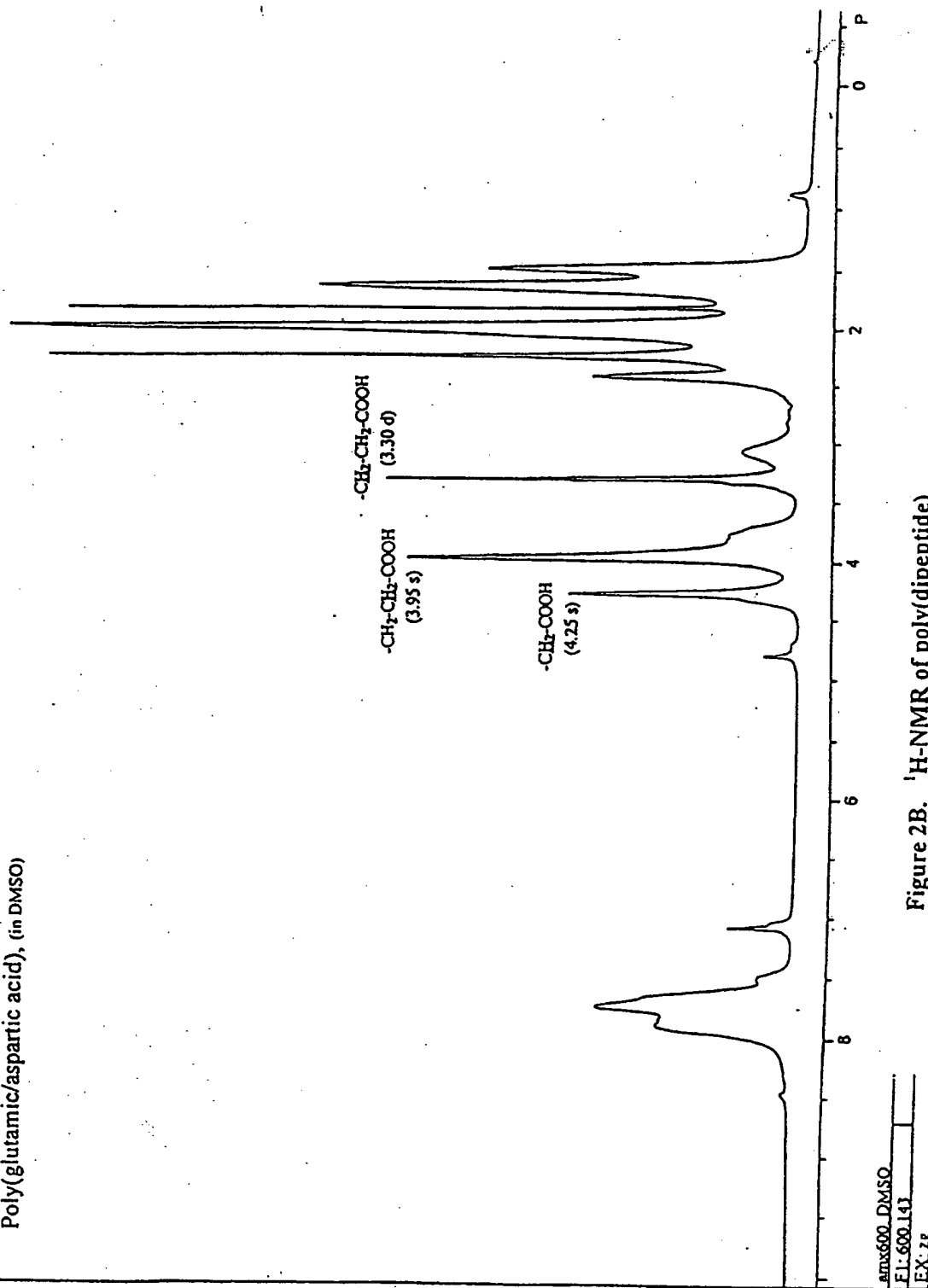
Figure 2B. ¹H-NMR of poly(dipeptide)

Figure 2C.   ¹H-NMR of paclitaxel,(in DMSO)

Conjugate paclitaxel, (in H2O)

-CH₂-CH₂-COO-
(4.32 s)

-CH₂-CH₂-COO-
(3.70 d)

-CH₂-COO-
(4.63 s)

C₂·-H (4.91 d)

PPM

Figure 2D. ¹H-NMR of poly(dipeptide)-paclitaxel conjugates

amx600 D20
F1 600.141
EX x.pur
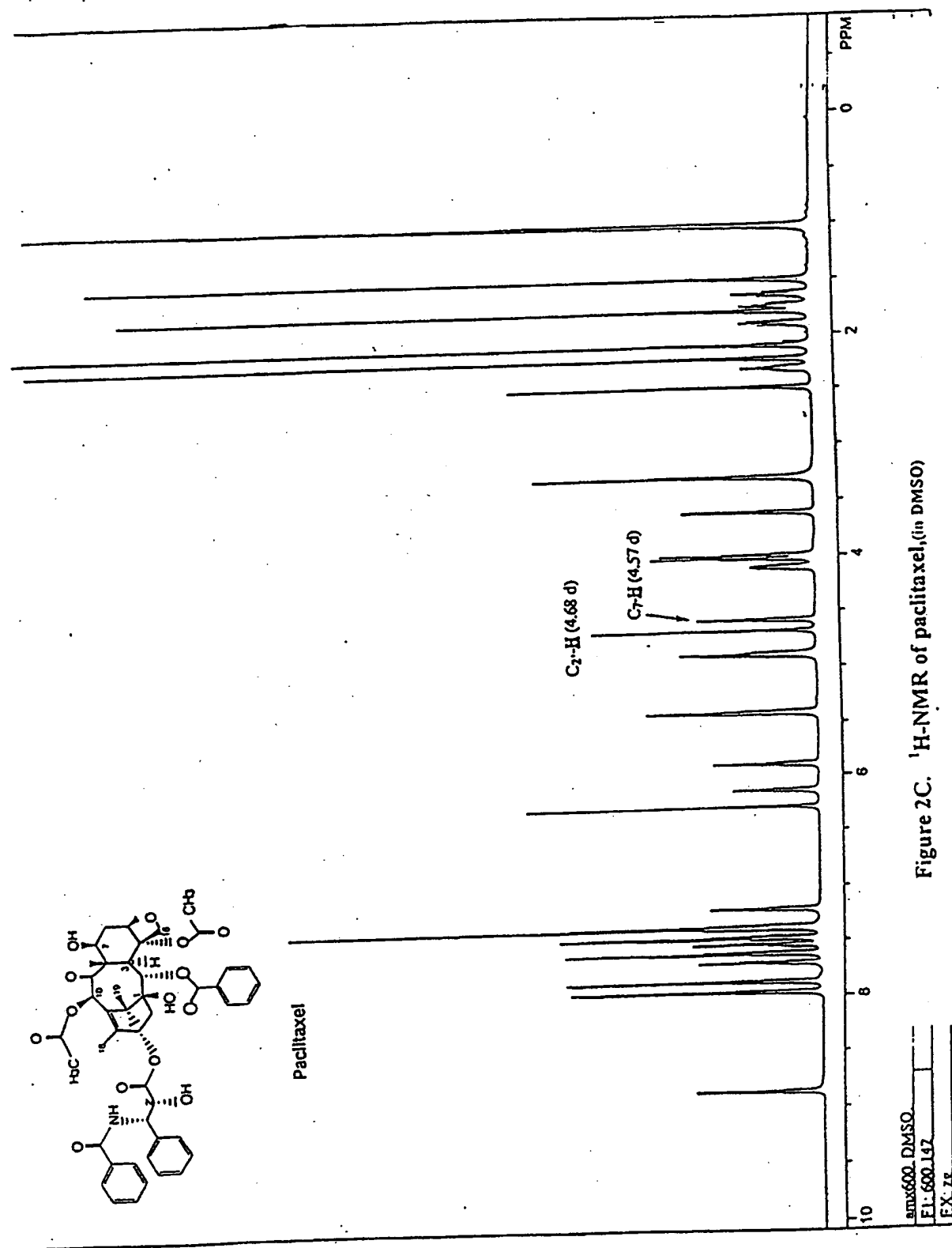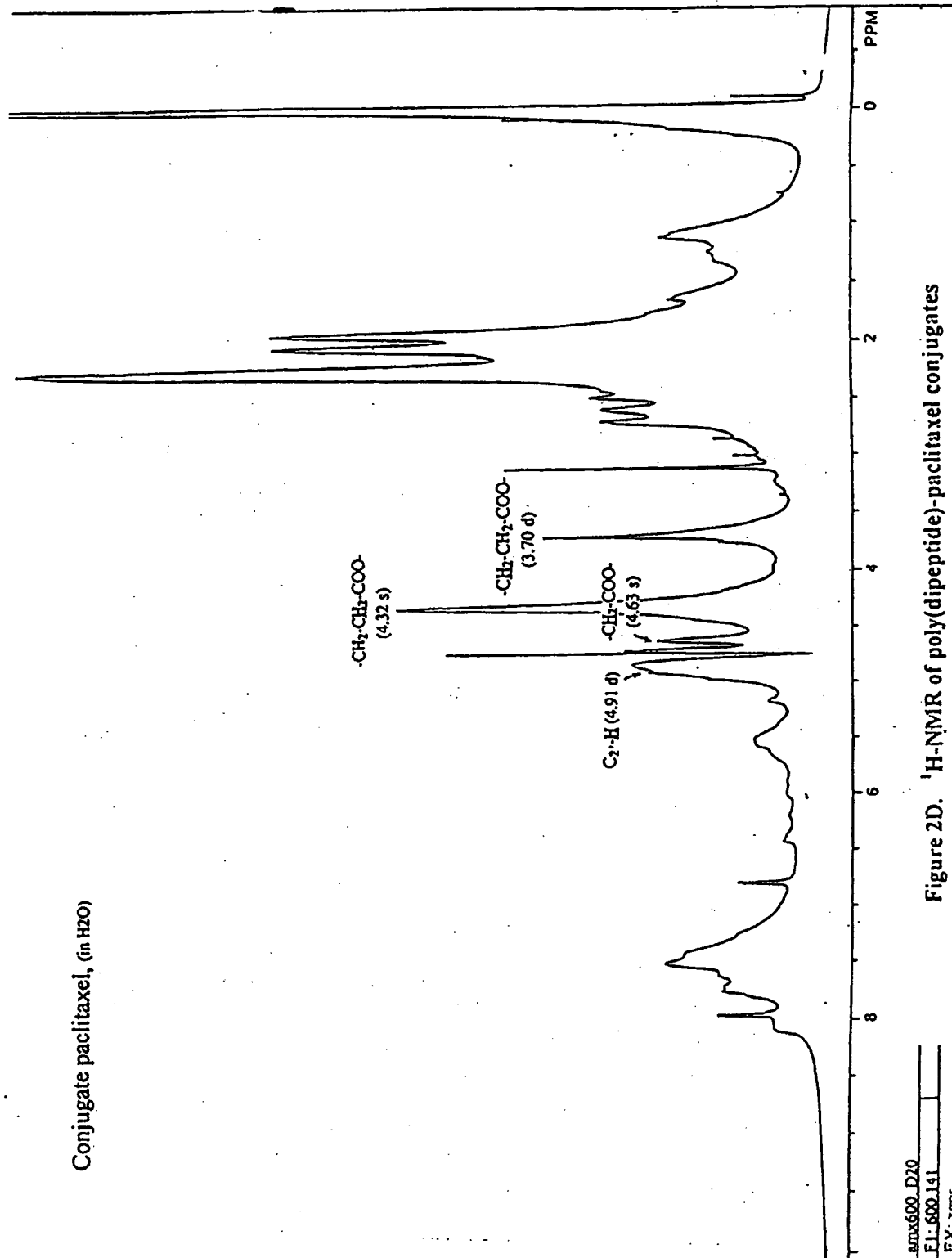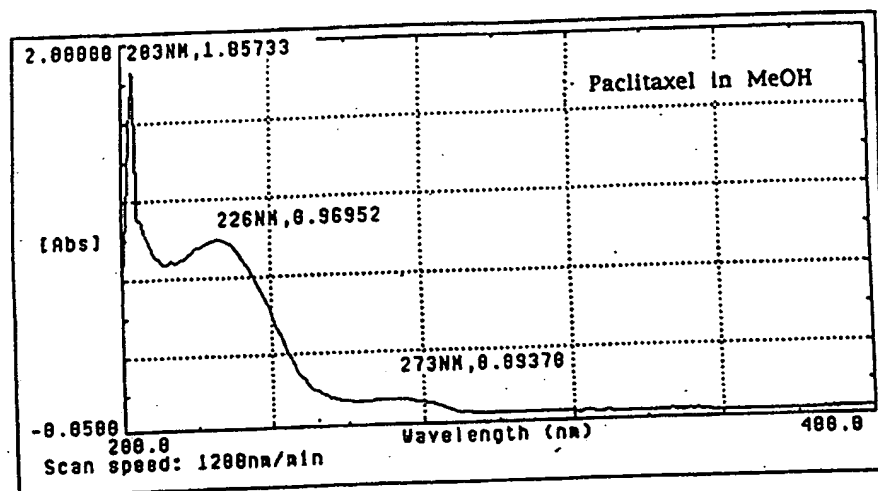
Figure 3A.
UV scan of paclitaxel
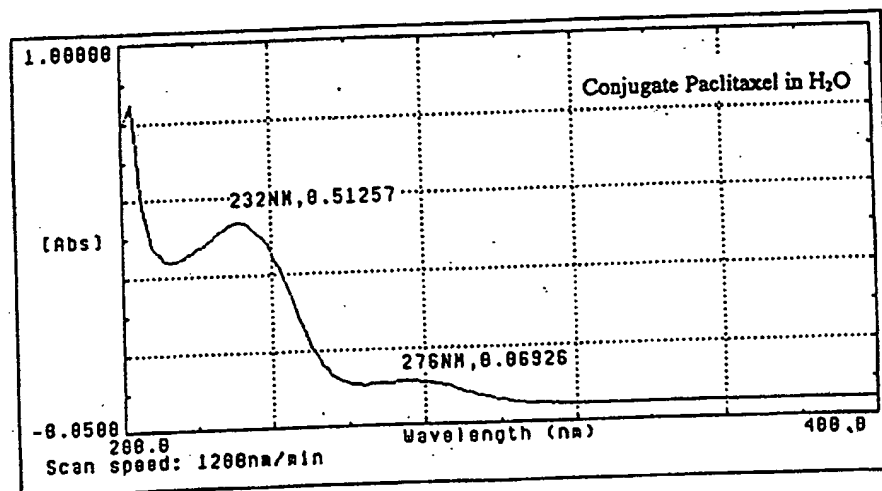
Figure 3B.
UV scan of poly(dipeptide)-paclitaxel, (poly(glutamic/aspartic acid)-paclitaxel conjugate)

Conjugate Paclitaxel in H_2O

[Abs]

239NM,0.47400

270NM,0.05230

1.00000

-0.0500

200.0   Wavelength (nm)   400.0

Scan speed: 1200nm/min
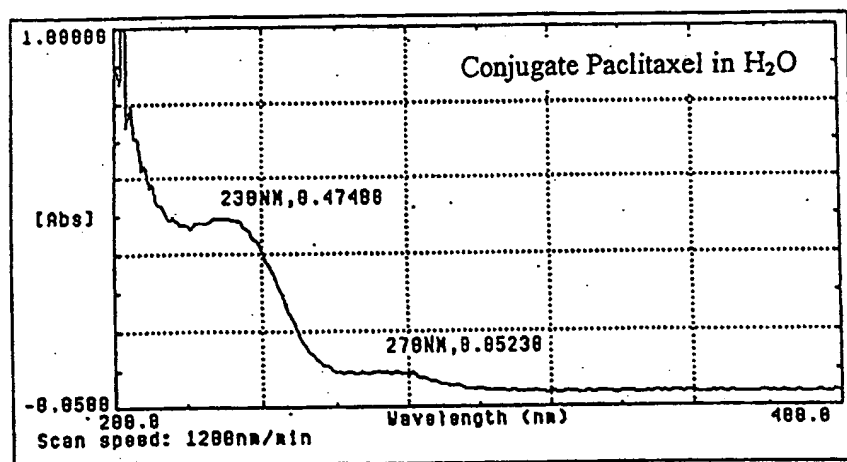
Figure 4A.
UV scan of poly(glutamic/aspartic acid)-paclitaxel conjugate

1.00000

Poly(dipeptide) in H_2O

[Abs]

-0.0500

200.0   Wavelength (nm)   400.0

Scan speed: 1200nm/min   λ:230.00   Reading:0.04975
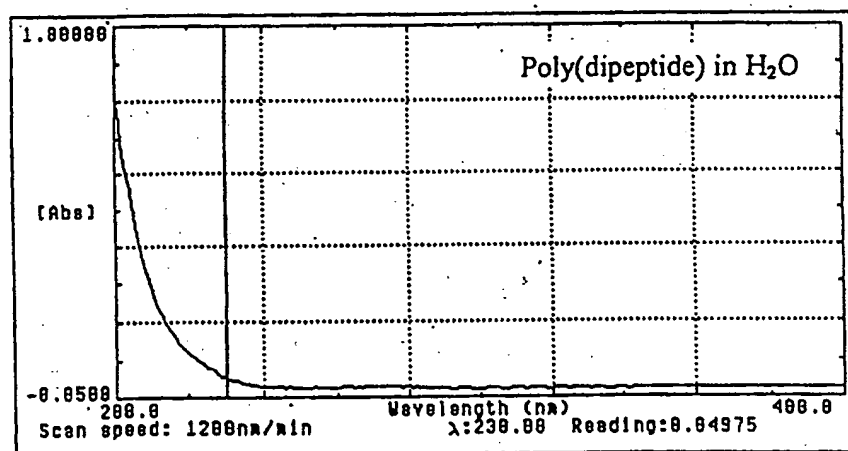
Figure 4B.
UV scan of poly(glutamic/aspartic acid)

The Curve of Paclitaxel Standard (228nm)

$y = 1.0575e\text{-}2 + 3.7473e\text{-}2x \quad R^{\wedge}2 = 0.999$
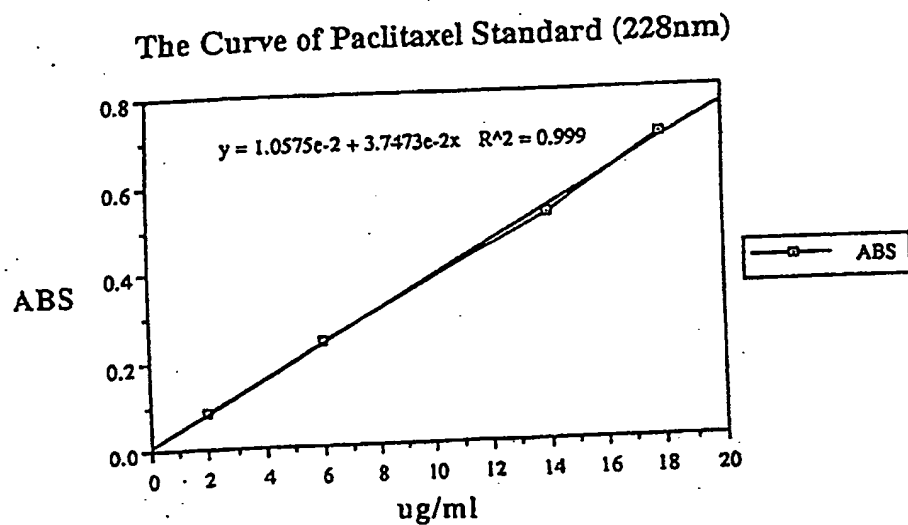
Figure5A. Standard UV absorption (228nm) of Paclitaxel
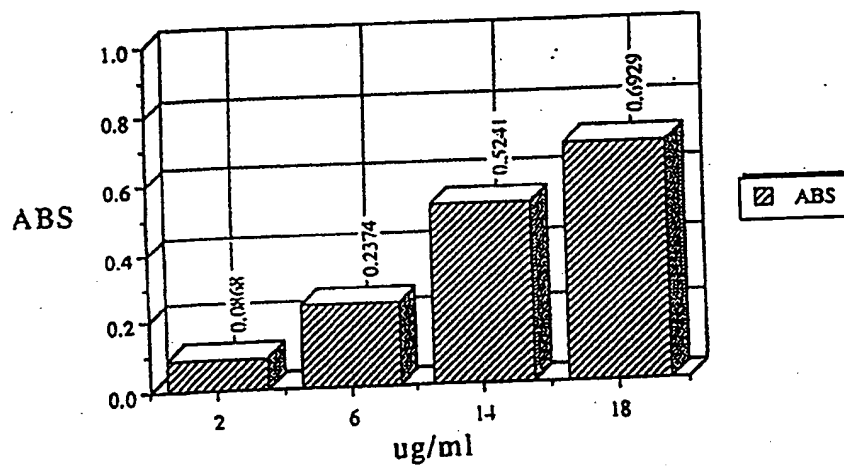
Figure5B. Standard UV absorption (228nm) of Paclitaxel
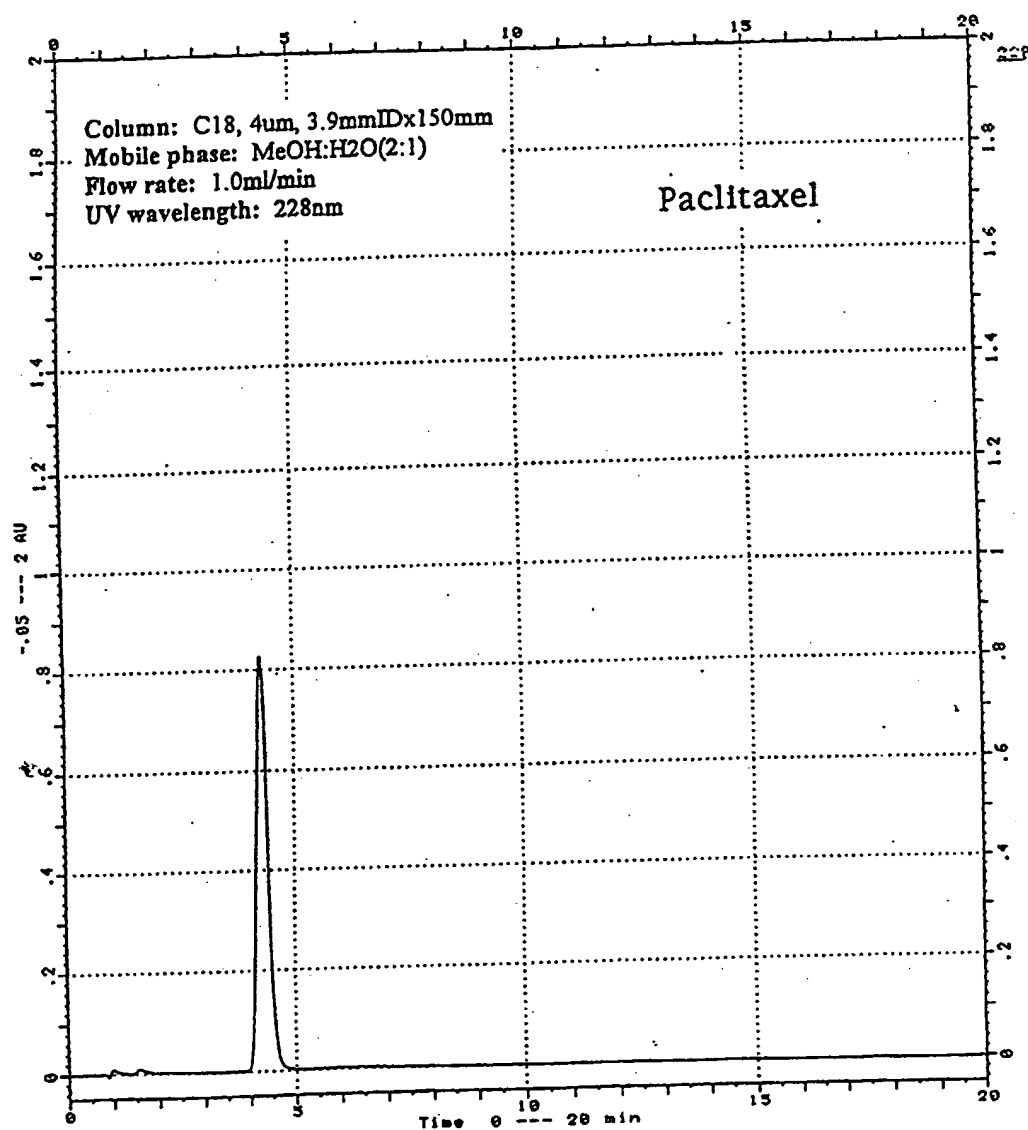
Figure 6.
HPLC analysis of paclitaxel
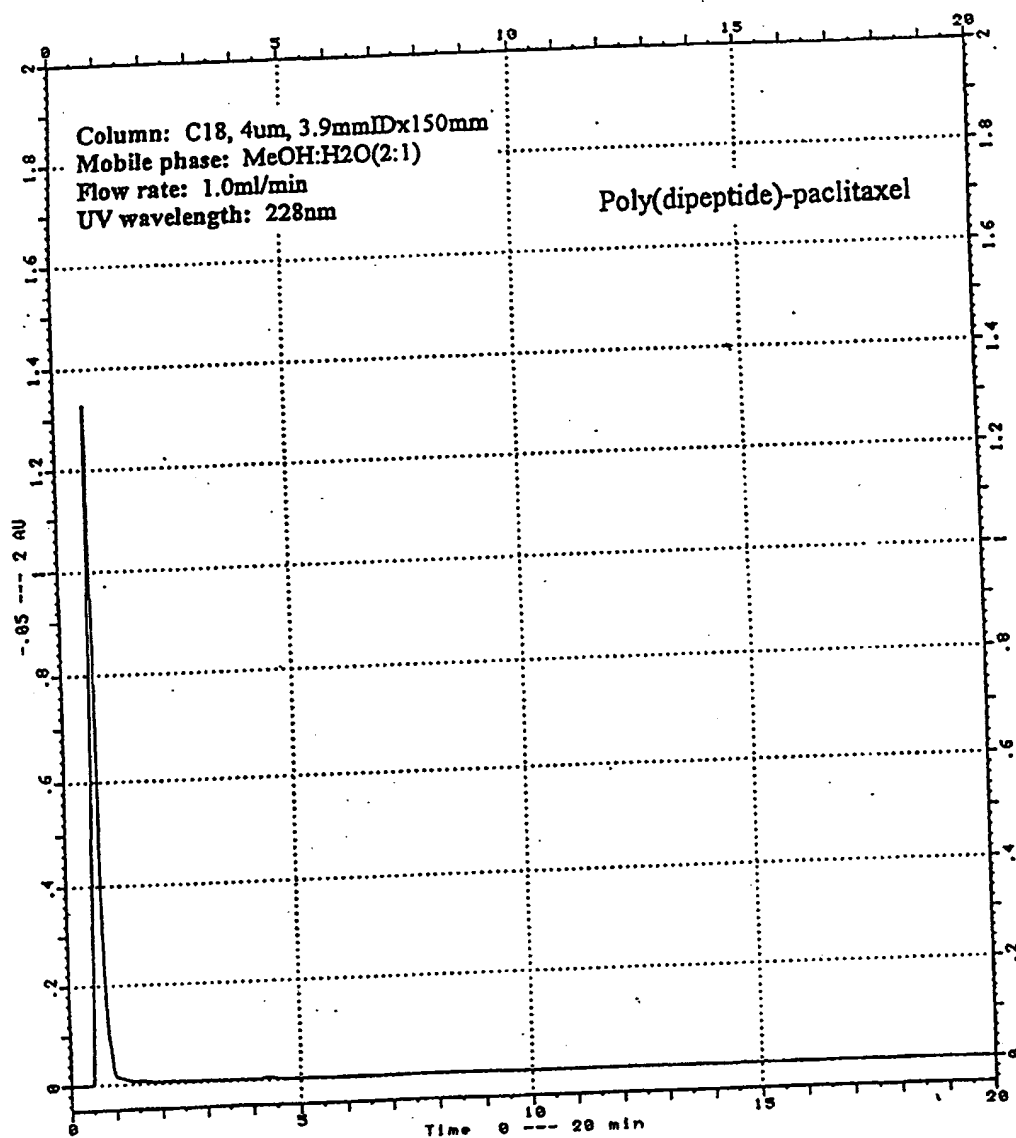
Figure 7.
HPLC analysis of poly(dipeptide)-paclitaxel conjugate
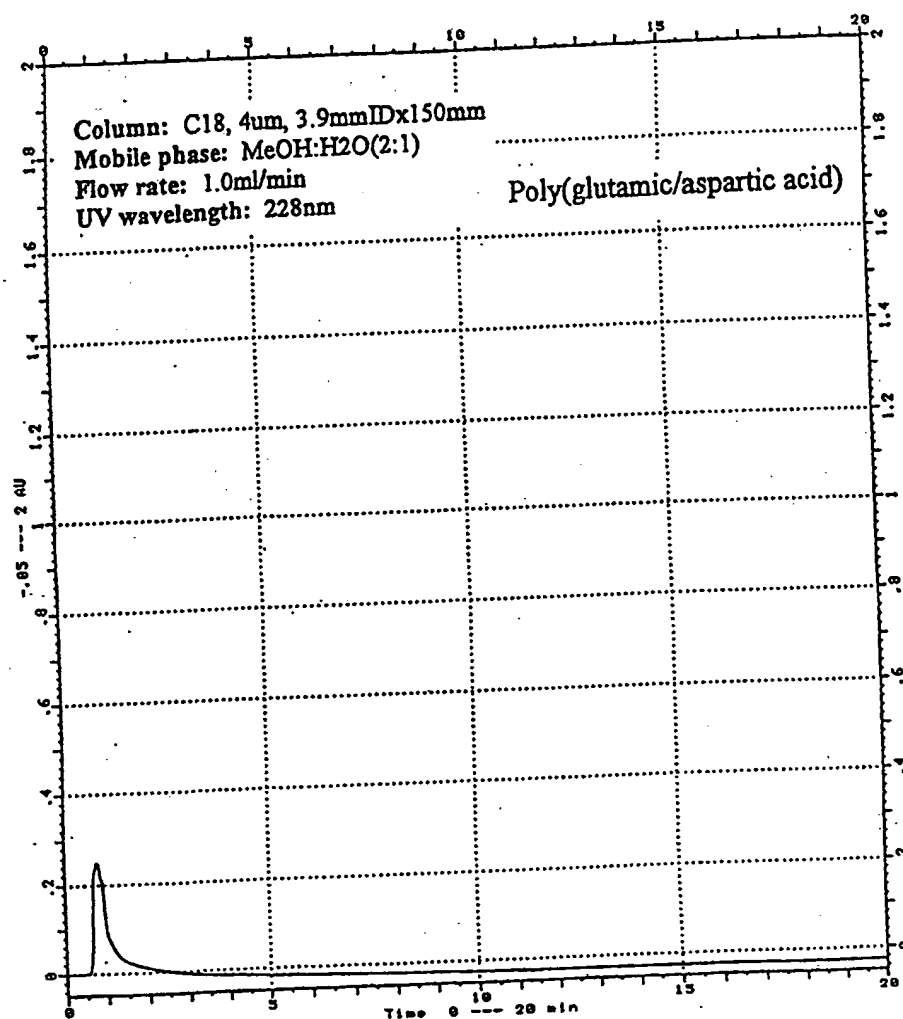
Figure 8.
HPLC analysis of poly(glutamic/aspartic acid)

Poly(dipeptide)-paclitaxel + Paclitaxel

Column: C₁₈, 4um, 3.9mmIDx150mm
Mobile phase: MeOH:H₂O(2:1)
Flow rate: 1.0ml/min
UV wavelength: 228nm
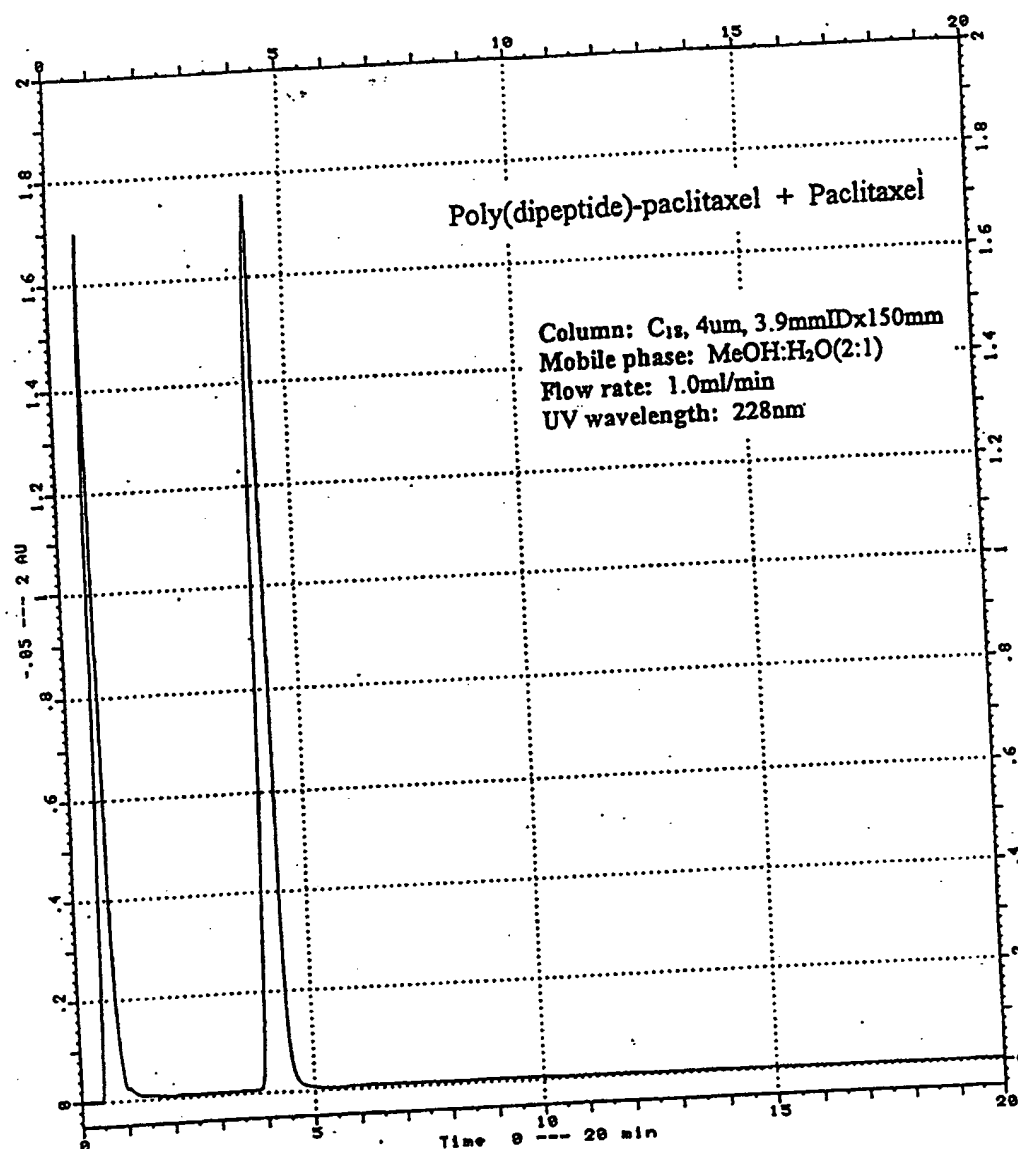
Figure 9A.
HPLC chromatogram of a mixture of poly(dipeptide)-paclitaxel and
unconjugated paclitaxel
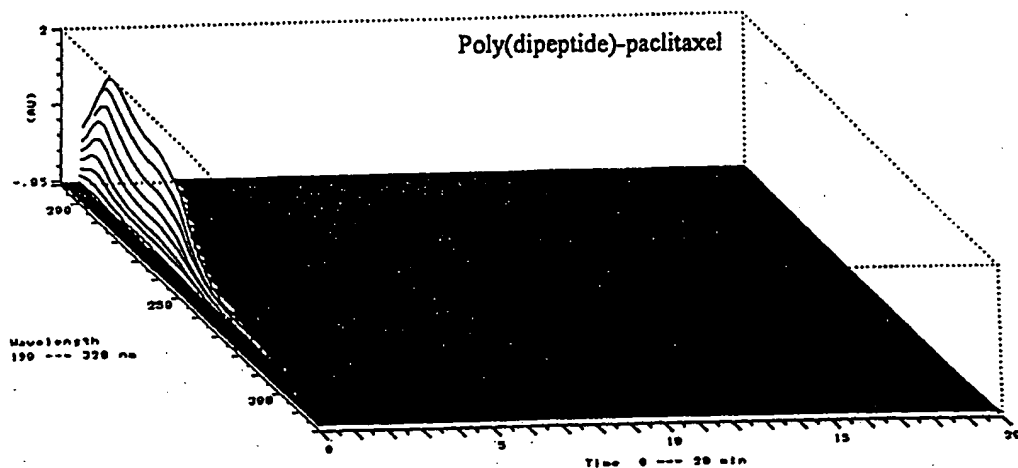
Figure 9B.
HPLC 3D chromatogram of poly(dipeptide)-paclitaxel conjugate
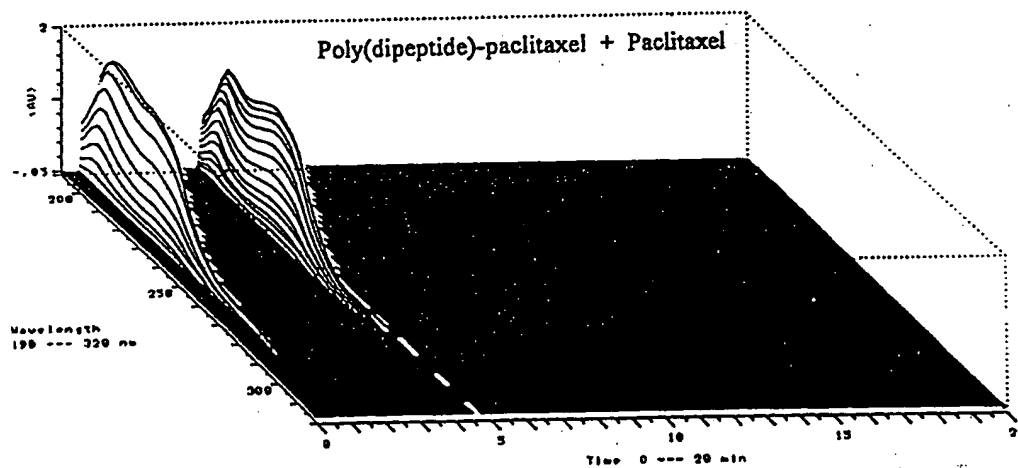
Figure 9C.
HPLC 3D chromatogram of a mixture of poly(dipeptide)-paclitaxel and
unconjugated paclitaxel

Release of Paclitaxel from Poly(dipeptide)-paclitaxel
Conjugate in PBS at PH 7.4
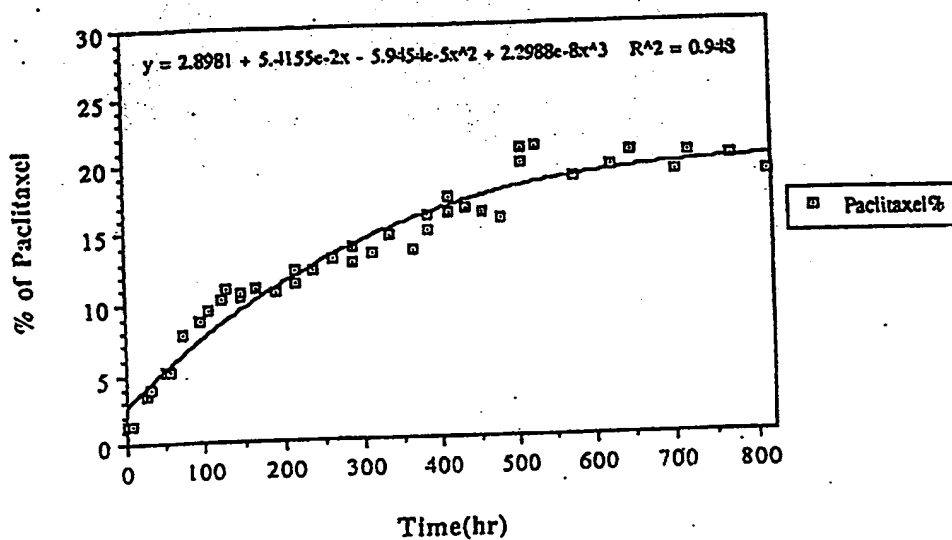
Figure 9D.
Sustained release properties of poly(dipeptide)-paclitaxel in PBS at PH
7.4

Stability of Poly(dipeptide)-paclitaxel Conjugate
in 10mM PBS at PH 7.4

$$K = \frac{2.303}{t} \log \frac{C_0}{C} = \frac{2.303}{814 hrs} \log \frac{98.623\%}{81.225\%} = 2.3847 \times 10^{-4} hrs$$

Half Life

$$t_{1/2} = \frac{2.303}{K} \log \frac{C_0}{\frac{C_0}{2}} = \frac{2.303}{K} \log 2 = \frac{0.693}{K} = \frac{0.693}{2.3847 \times 10^{-4} hrs} = 2906.0 hrs = 121.08 \ days$$

Useful Life

$$t_{1/10} = \frac{2.303}{K} \log \frac{100}{10} = \frac{2.303}{K} = \frac{0.1054}{K} = \frac{0.1054}{2.3847 \times 10^{-4} hrs} = 441.98 hrs = 18.4 \ days$$

Figure 9E.
Useful half-life determination of poly(dipeptide)-paclitaxel conjugate

Effect of Paclitaxel Conjugate and Paclitaxel on Human
Nasopharyngeal Cancer Cells(KB) in vitro

Effect of Paclitaxel Conjugate and Paclitaxel on Human
Breast Cancer Cells(MDA MB 231) in vitro

Effect of Paclitaxel Conjugate and Paclitaxel on Human
Prostate Cancer Cells(PC3) in vitro
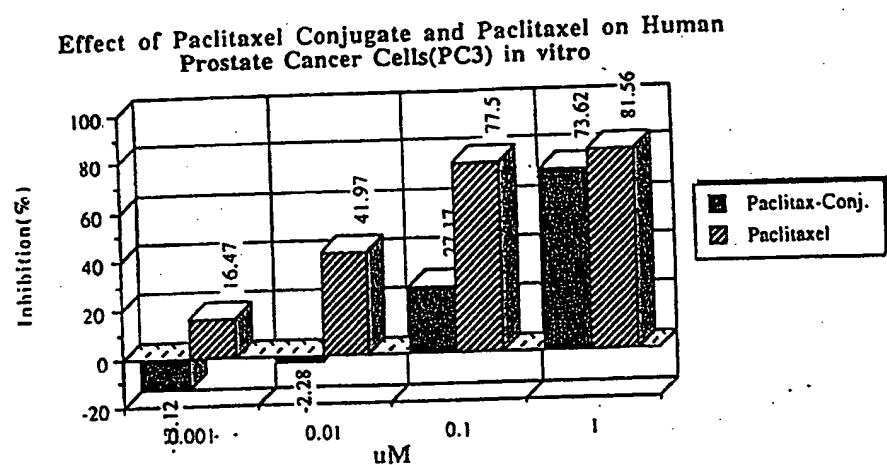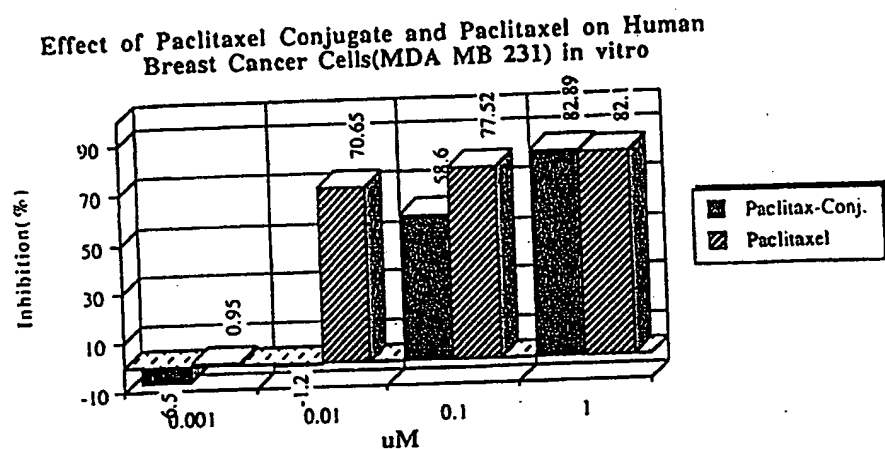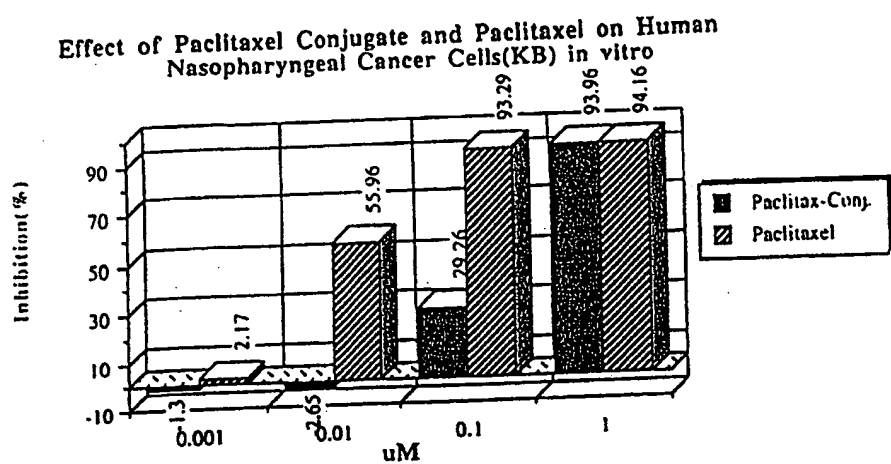
Figure 9F.

Effects of poly(dipeptide)-paclitaxel conjugate and unconjugated paclitaxel
in vitro cell culture

Effects of Paclitaxel conjugated and paclitaxel on human prostate cancer cells(PC3) in vitro
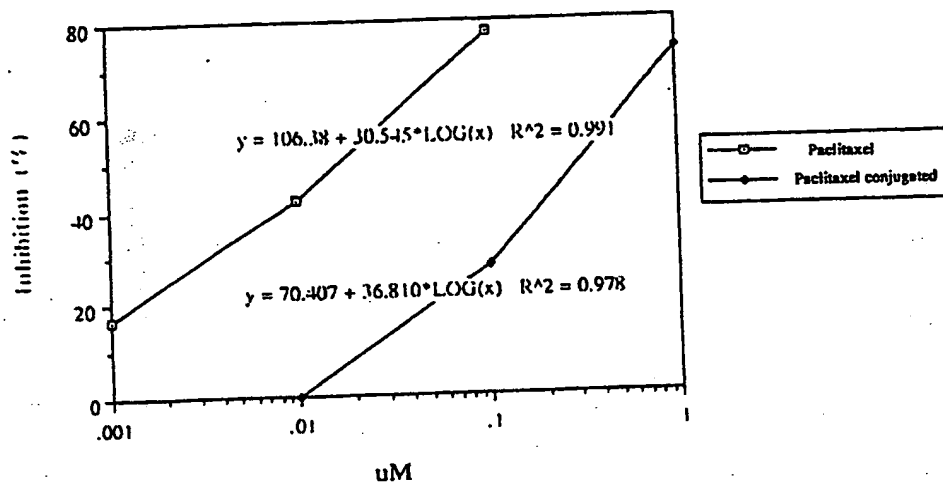
$IC_{50}(\text{poly(dipeptide)-paclitaxel}) = 0.279\mu M$

$IC_{50}(\text{paclitaxel}) = 0.0143\mu M$

Figure9G. Cytotoxicity(IC_{50}) of poly(dipeptide)-paclitaxel conjugated and unconjugated paclitaxel on human prostate cancer cells(PC3) in vitro
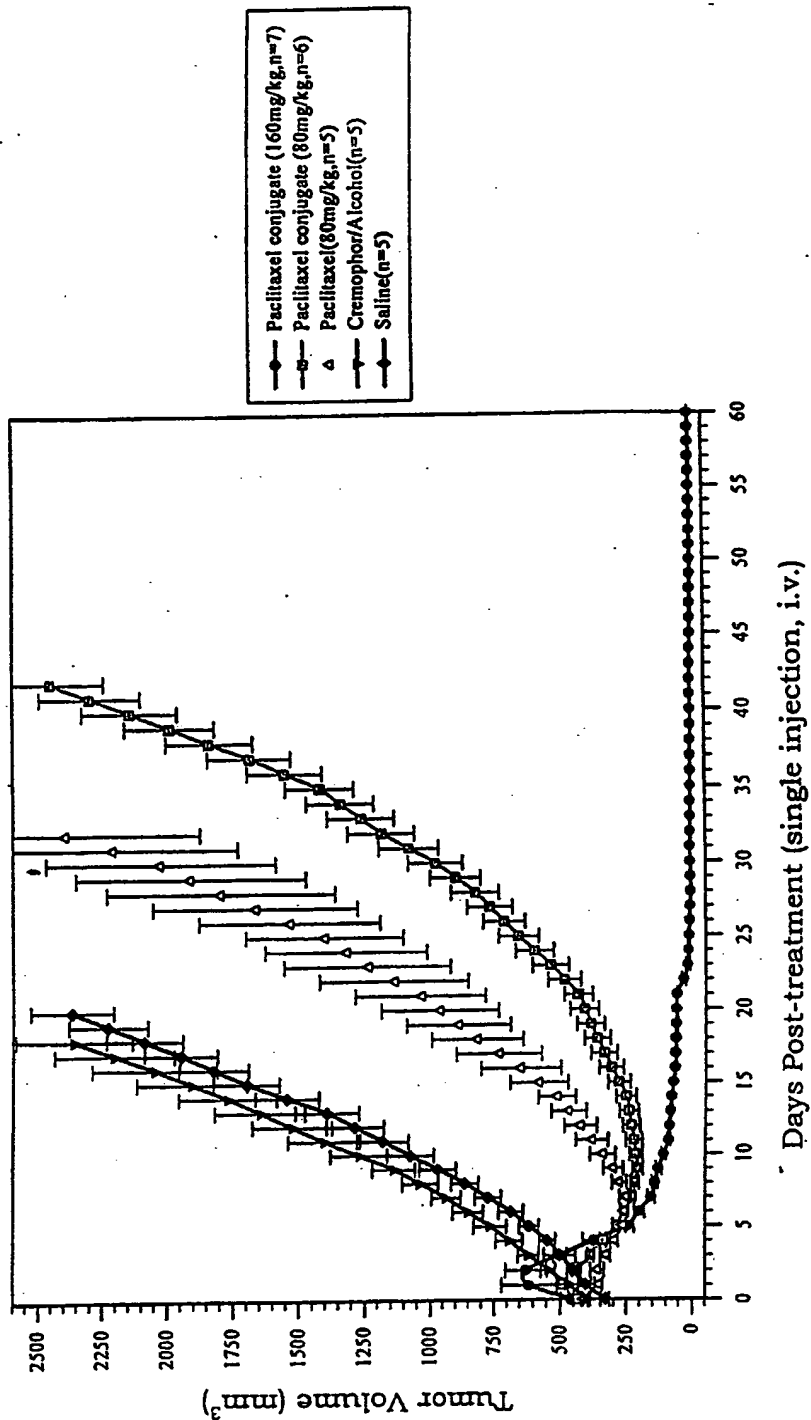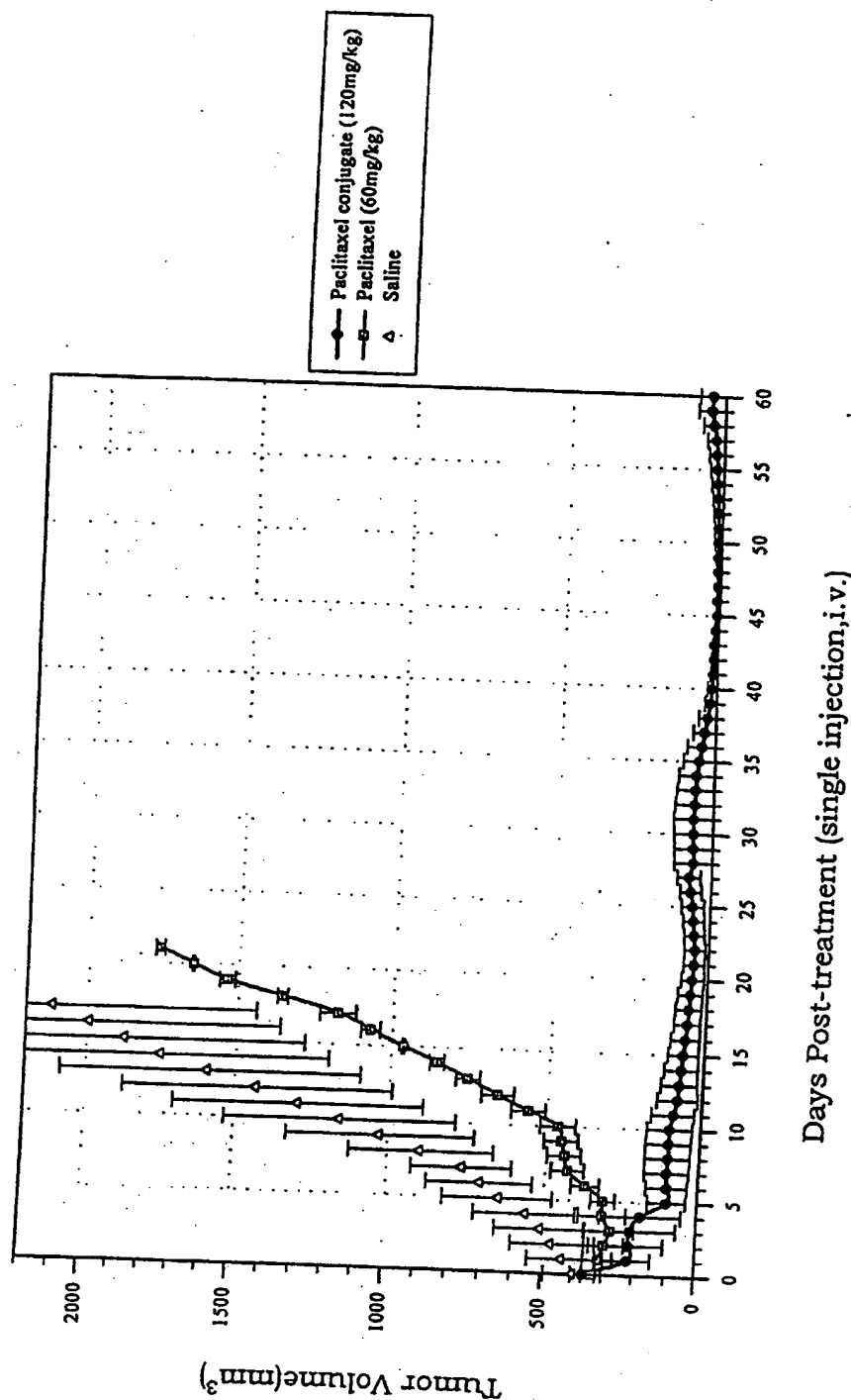
Figure 10. In vivo anti-tumor activity of poly(dipeptide)-paclitaxel conjugate and unconjugate paclitaxel
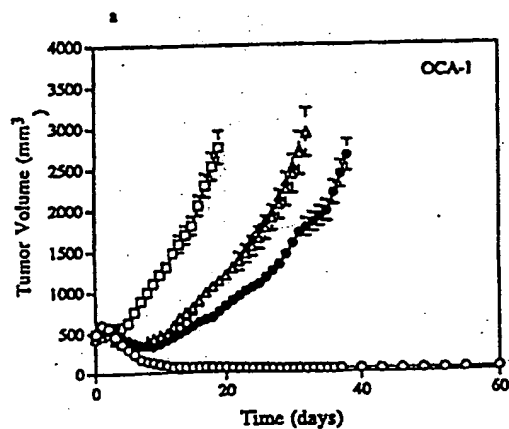
Fig. 2. Antitumor effect of PG-TXL and paclitaxel on mice bearing murine ovarian tumor OCA-1 in a single dose. Data are presented as means of tumor volumes; bars, SD. In a, mice bearing OCA-1 tumor received injections of: □ PG control (800 mg/kg; n = 9); ▲ paclitaxel (80 mg/kg; n = 7); △ paclitaxel (80 mg/kg) plus PG (800 mg/kg; n = 5); ●, PG-TXL (80 mg equivalent paclitaxel/kg; n = 6); or ○, PG-TXL (160 mg equivalent paclitaxel/kg; n = 26).

Figure 11.
In vivo antitumor activity of polyglutamic acid-paclitaxel
(PG-Paclitaxel) and paclitaxel

Figure 12. In vivo anti-tumor activity of poly(dipeptide)-paclitaxel conjugate and unconjugate paclitaxel

Effect of Poly(dipeptide)-paclitaxel Conjugate on Nude Mice
Bearing Human Prostate Cancer (MDA PCa 2b)

Figure 13A. In vivo anti-tumor activity of poly(dipeptide)-paclitaxel conjugate and unconjugate paclitaxel

Effect of PG-Taxol and Taxol on Nude Mice
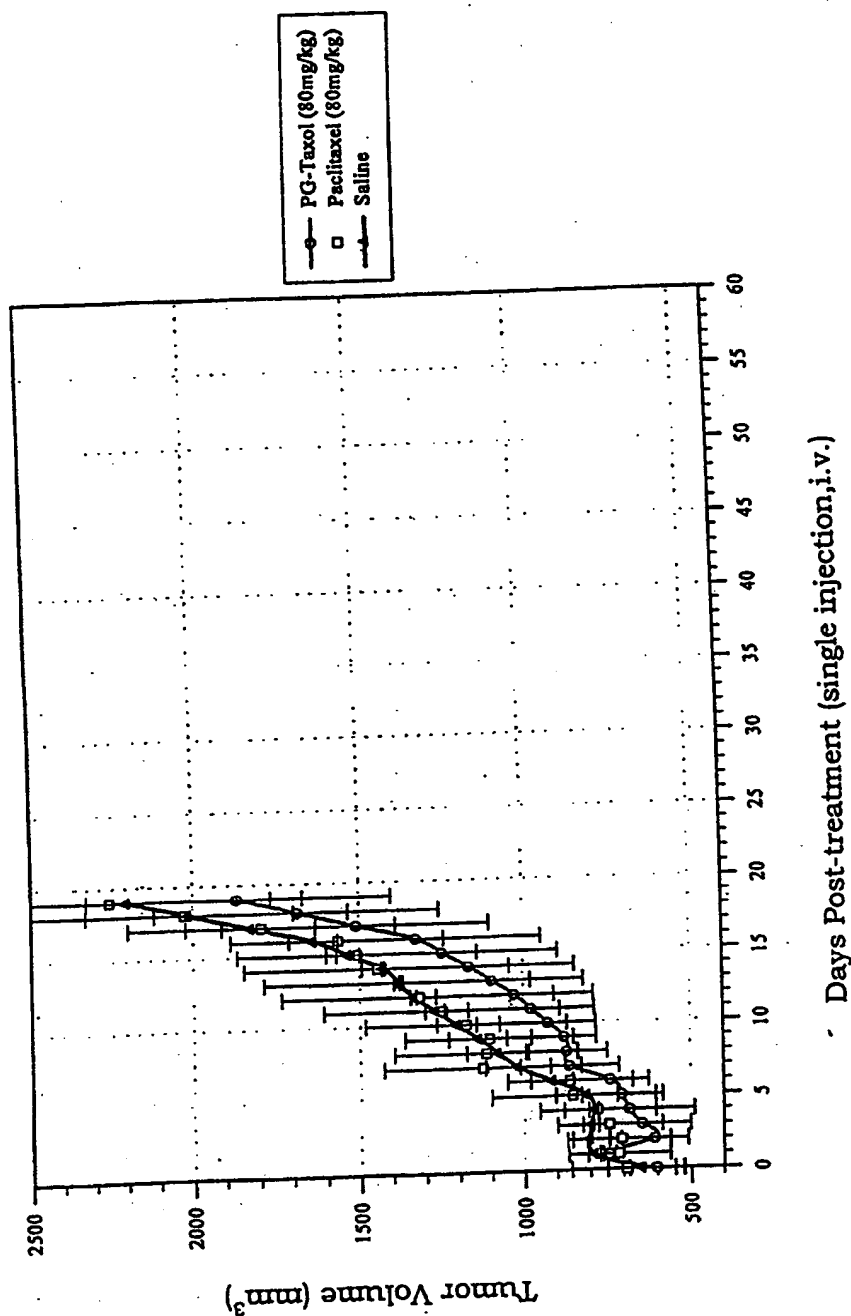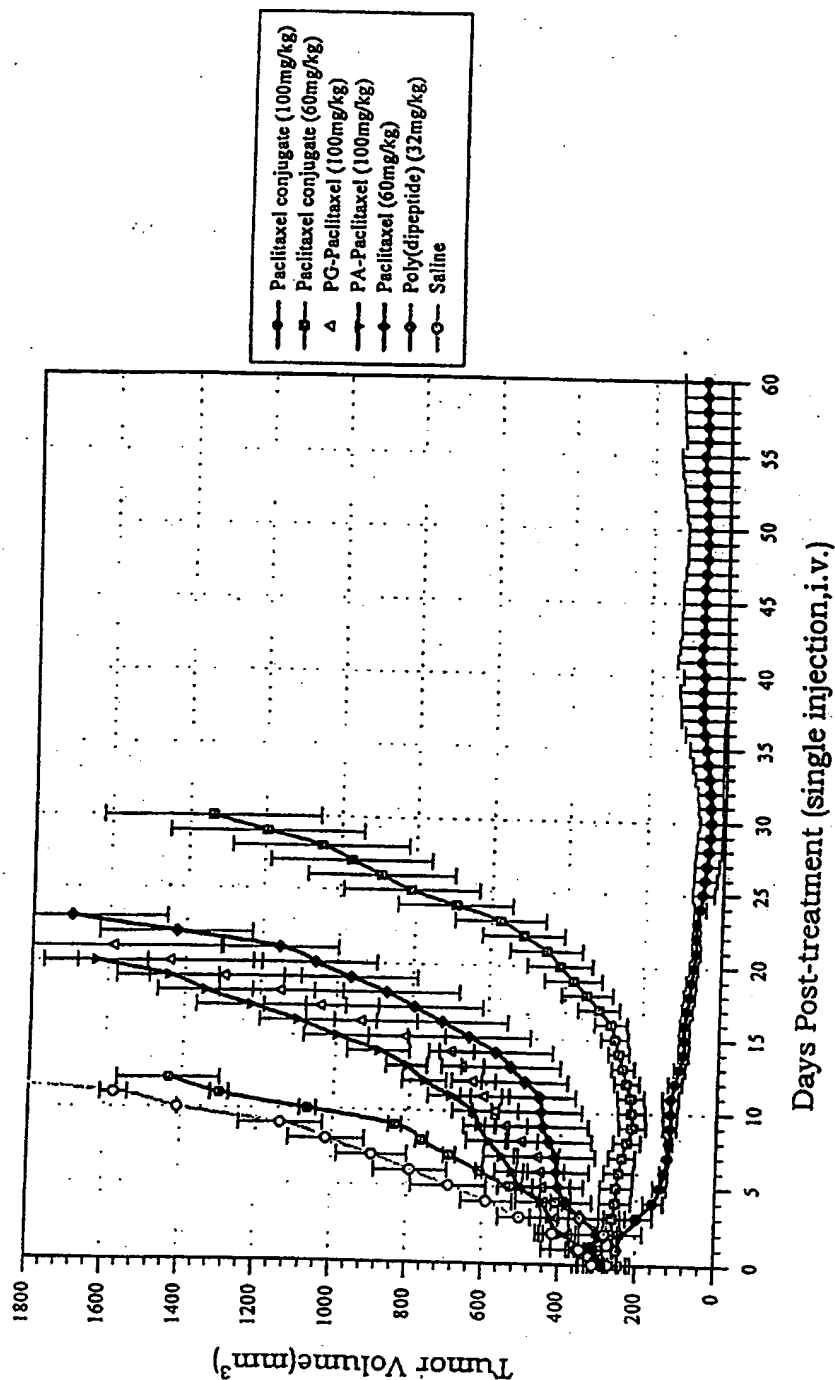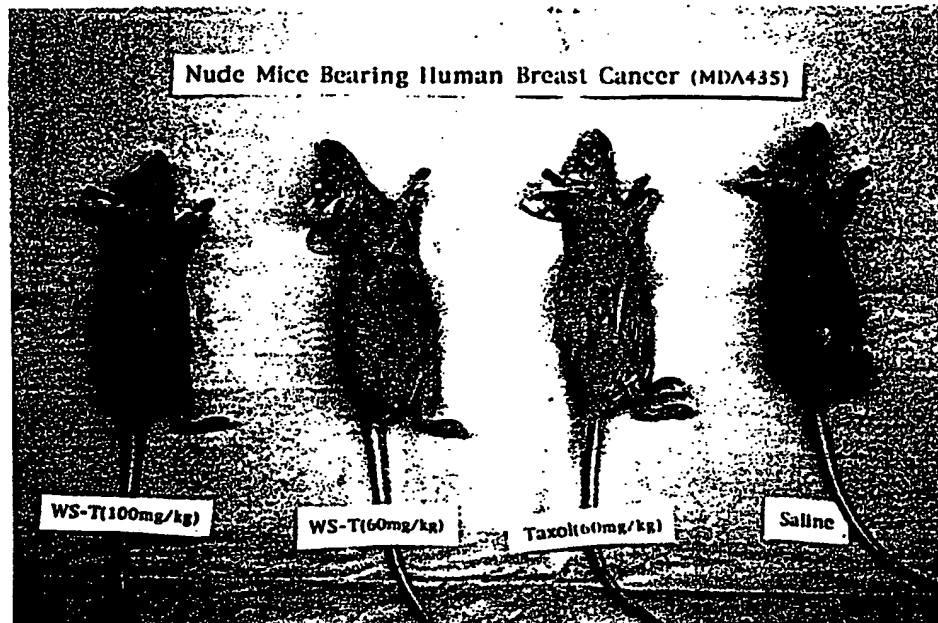Bearing Human Prostate Cancer (MDA PCa 2b)

Figure 13B. In vivo anti-tumor activity of polyglutamate-paclitaxel conjugate(PG-Taxol) and unconjugate pac

Effect of Poly(dipeptide)-paclitaxel Conjugate on Nude Mice
Bearing Human Prostate Cancer (PC3)

Figure 14. In vivo anti-tumor activity of poly(dipeptide)-paclitaxel conjugate polyglutamate-paclitaxel conjugate(P Paclitaxel), polyaspartate-paclitaxel(PA-Paclitaxel) and unconjugate paclitaxel

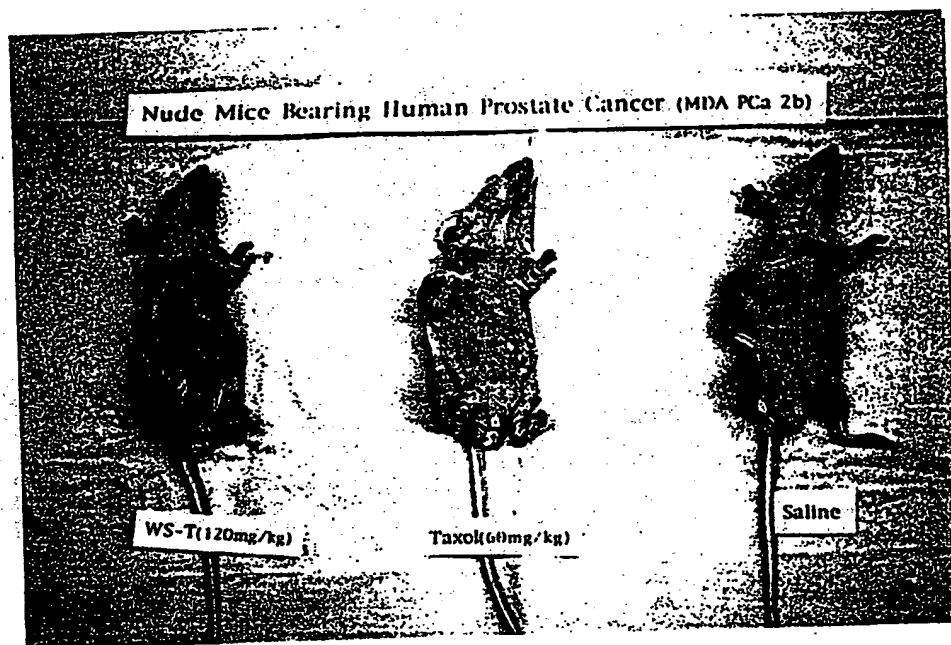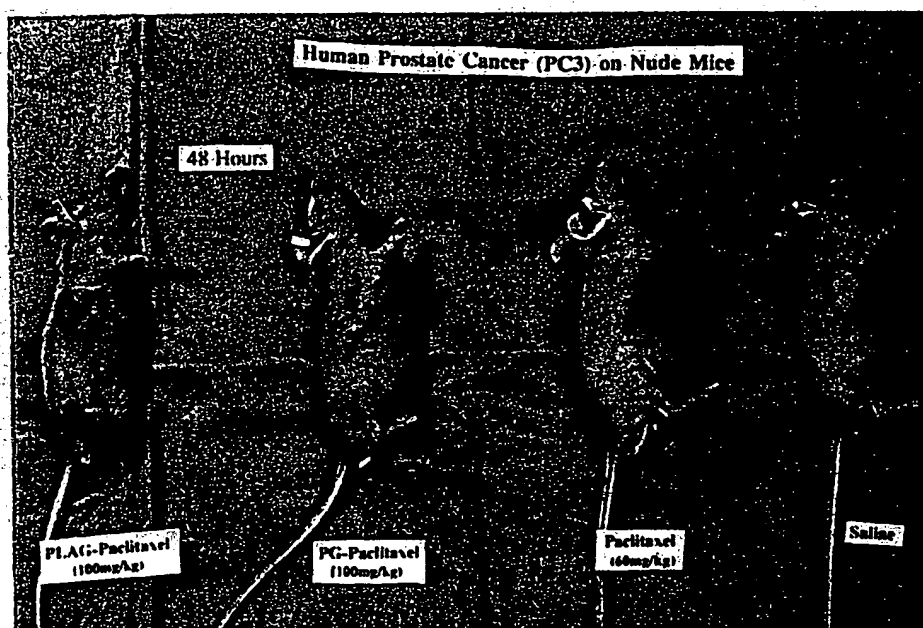Mice at 15 days post-treatment

Figure 15A.

Anti-tumor activity of poly(dipeptide)-paclitaxel conjugate(WS-T) and unconjugate paclitaxel(Taxol) on athymic nude mice bearing human breast tumor

Human Breast Cancer (MDA 435) on Nude Mice

Saline   Paclitaxel
(60mg/kg)   WS-Paclitaxel
(60mg/kg)   WS-Paclitaxel
(100mg/kg)

Mice at 43 days post-treatment

Figure 15B.

Anti-tumor activity of poly(dipeptide)-paclitaxel conjugate(WS-Paclitaxel) and unconjugate paclitaxel on athymic nude mice bearing human breast tumor

Mice at 15 days post-treatment

Figure 16.

Anti-tumor activity of poly(dipeptide)-paclitaxel conjugate(WS-T) and unconjugate paclitaxel(Taxol) on athymic nude mice bearing human prostate cancer
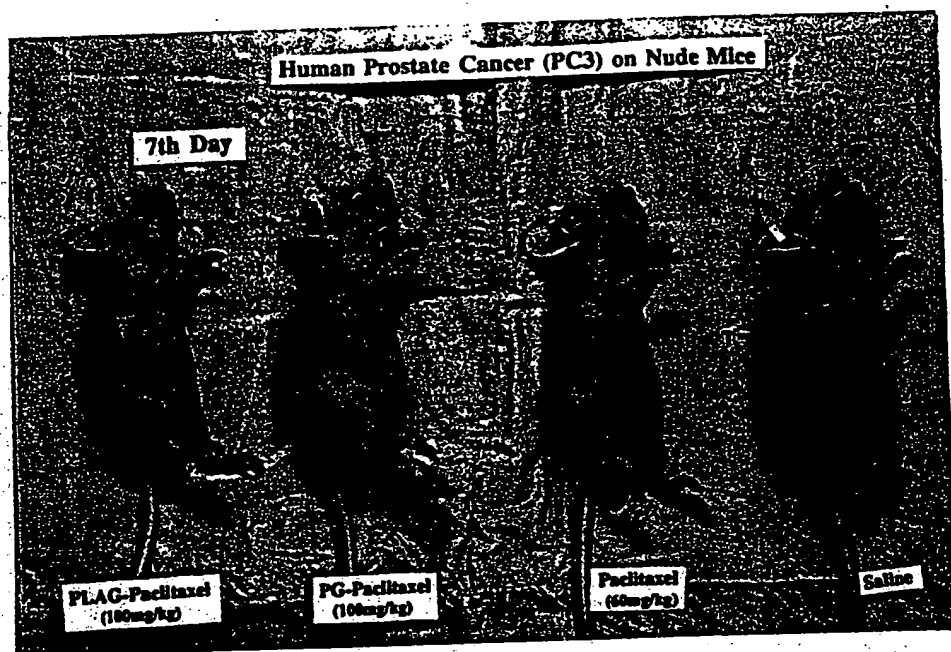
Mice at 48 hours post-treatment

Figure 17A.

Anti-tumor activity of poly(dipeptide)-paclitaxel conjugate(PLAG-Paclitaxel),
polyglutamate-paclitaxel(PG-Paclitaxel) and unconjugate paclitaxel on athymic
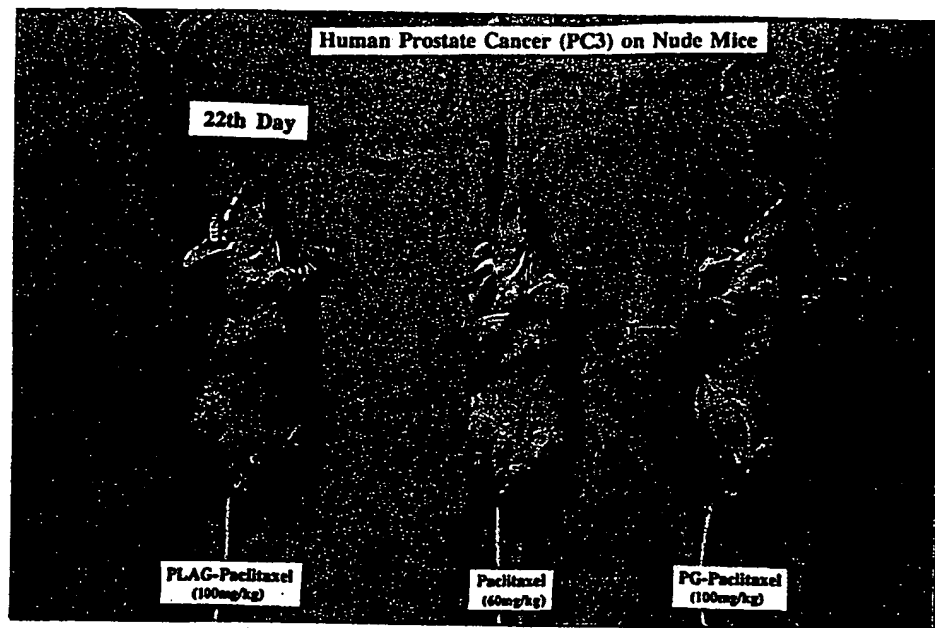nude mice bearing human prostate cancer

Mice at 7 days post-treatment

Figure 17B.

Anti-tumor activity of poly(dipeptide)-paclitaxel conjugate(PLAG-Paclitaxel),

polyglutamate-paclitaxel(PG-Paclitaxel) and unconjugate paclitaxel on athymic
nude mice bearing human prostate cancer

Mice at 22 days post-treatment

Figure 17C.

Anti-tumor activity of poly(dipeptide)-paclitaxel conjugate(PLAG-Paclitaxel),
polyglutamate-paclitaxel(PG-Paclitaxel) and unconjugate paclitaxel on athymic
nude mice bearing human prostate cancer